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THE EFFECT OF ORAL ADMINISTRATION OF HORMONES ON GROWTH RATE AND DEPOSITION IN THE CARCASS OF FATTENING STEERS [1]

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NUMEROUS investigations have shown the growth-stimulatory effect of natural and synthetic hormones when administered to cattle (Andrews *et al.*, 1954; and Dinusson *et al.*, 1950) and lambs (Andrews *et al.*, 1953, 1949; Means *et al.*, 1953; and Perry *et al.*, 1951). In addition such physiological and anatomical responses as increased feed efficiency, altered sexual behavior, mammary stimulation, pelvic changes and reduced carcass quality have been reported in both females and castrate males. Burroughs *et al.* (1954) reported growth stimulation from oral administration of stilbestrol to cattle, but concluded that it was without any of the undesirable side effects observed with pellet implantation.

The trial reported in this paper was designed to study the effect of administering diethylstilbestrol, dienestrol diacetate or hexestrol (dihydrodiethylstilbestrol) in the feed, in terms of growth rate, feed utilization, carcass quality, and estrogenic activity of the edible carcass. Technically diethylstilbestrol, dienestrol diacetate and hexestrol are classified as hormone-like chemicals, however for ease of expression these products will hereafter be referred to as hormones in this paper.

Experimental Procedure

Forty 2-year-old Hereford steers averaging 870 lb. liveweight were divided into four lots of ten steers, each, on the basis of liveweight and previous wintering gain. The four lots were fed a fattening ration of ground corn, ground corn cobs, 3.5 lb. Purdue Cattle Supplement A—1000 lb. mix, soybean meal 650.5 lb., molasses 140.0 lb., alfalfa meal 140.0 lb., bonemeal 52.0 lb., salt with cobalt 17.0 lb., Vitamin A and D concentrate containing 4,540,000 U.S.P. units Vitamin A and 567,500 U.S.P. units Vitamin D per pound, 0.5 lb.—and free choice minerals (2 bonemeal : 1 salt plus loose salt).

[1] Contribution from the Department of Animal Husbandry, Journal Paper No. 827.
[2] Lafayette, Indiana.

The following treatments were administered:

Lot I —*control*—ration as listed above.

Lot II —*Stilbestrol*—10 mg. diethylstilbestrol per animal daily in Supplement A.

Lot III—*dienestrol*—10 mg. dienestrol per animal daily in Supplement A.

Lot IV —*hexestrol*—10 mg. hexestrol per animal daily in Supplement A.

The hormones were dissolved in corn oil. Five hundred ml. corn oil were used to dissolve the hormones for a 500 lb. mixture. At slightly elevated temperatures (100° C.) the hormones went into solution easily. The corn oil-hormone solution was mixed with a small amount of Supplement A in a tub—rubber gloves were used—and then this pre-mix was dumped into the vertical mixer as a part of a 500 lb. portion of Supplement A.

The fattening trial extended over a 123-day period. At the completion of the trial, the cattle were retained for one week prior to slaughtering. Five of the animals in each treated lot, selected at random, were removed from the hormone treatment whereas the remaining five in each treated lot continued to receive their respective hormone treatments until the day prior to slaughter.

At time of slaughter, one pound of neck meat was obtained from each steer for hormone assay. The meat was ground to a hamburger-like fineness, air dried and mixed with a commercial poultry mash. This meat-mash mixture was fed to castrated, female mice of the Rockland-Swiss strain for ten days. At autopsy, the uteri were removed and weighed on a torsion balance. The presence of residual hormone was detected by differences in the average uterine weights of animals fed control meat in comparison to those fed meat from animals fed hormones. (Stob *et al.*, 1954).

Results and Discussion

Effect of Oral Hormones on Growth Rate

The feeding of 10 mg. daily per steer of either stilbestrol (Lot II) or hexestrol (Lot IV) in a fattening ration resulted in highly significant increased growth rate. Over the 123-day fattening period, control steers receiving no supplemental hormone gained 274 lb. per animal whereas stilbestrol-fed or hexestrol-fed steers gained 324 lb. and 326 lb., respec-

tively, or 50 and 52 lb. more than the control animals. Dienestrol-fed steers (Lot III) gained slightly less rapidly than either the stilbestrol-fed or hexestrol-fed steers, but yet gained 39 lb. more ($P < 0.05$) than the control steers. (table 1.)

TABLE 1. THE EFFECT OF ORALLY ADMINISTERED HORMONES ON GROWTH RATE, FEED CONSUMPTION AND FEED UTILIZATION OF TWO-YEAR-OLD STEERS

(May 13 to September 13, 1954. 123 Days)

	Lot I Control	Lot II 10 mg. Stilbestrol	Lot III 10 mg. Dienestrol	Lot IV 10 mg. Hexestrol
No. per lot	10	10	10	10
		Growth Rate		
Av. initial wt., lb.	873	875	871	871
Av. final wt., lb.	1147	1199	1184	1197
Total gain, lb.	274	324**	313*	326**
Av. daily gain, lb.	2.33	2.64	2.55	2.65
		Feed Consumption		
Gr. corn, lb.	11.6	12.6	12.5	12.6
Gr. corn cobs, lb.	9.2	9.4	9.3	9.3
Suppl. A, lb.	3.5	3.5	3.5	3.5
Mineral, lb.	.03	.03	.04	.02
		Fed per Pound Gain		
Gr. corn, lb.	5.2	4.8	4.9	4.8
Gr. corn cobs, lb.	4.1	3.6	3.6	3.5
Suppl. A, lb.	1.6	1.3	1.4	1.3
Mineral, lb.	.01	.01	.01	.01

* L.S.M.D., 5%, 34 lb.
** L.S.M.D., 1%, 46 lb.

The rate of gain of the hormone-treated cattle varied markedly during the experimental period. The largest percent of the additional gains attributed to hormone administration occurred during the first 28 days of treatment. For example, the total gain per steer of the stilbestrol-treated cattle during the 123-day trial was 50 lb. greater than that of the controls. However, 27 lb. of the advantage in favor of the stilbestrol-fed steers was attained during the first 28 days of treatment. Similar observations were made with respect to hexestrol and dienestrol administration. This marked early difference could not be attributed to feed intake, since the hormone-treated animals consumed the same amount of feed per day as the control steers during the period involved. (table 2.)

Effect of Oral Hormones on Feed Consumption and Utilization

The intake of corn for the entire experimental period was approximately one pound per steer per day greater, in each of the hormone treated groups, than in the controls. There were no differences in roughage or mineral intake although both were constantly available (table 1). The level of Supplement A feeding was maintained at 3.5 lb. per animal, daily in all lots. Since the hormone-fed steers gained significantly more on slightly more feed than steers receiving no hormone, increased utilization of feed was apparent. The three hormone-fed lots required from 9 to 12 percent less feed per unit of gain than the control

TABLE 2. EFFECT OF ORALLY ADMINISTERED HORMONES ON GROWTH PER STEER BY 28-DAY PERIODS

	Steer No.					
	1	2	3	4	5[1]	Total
Lot I	77	69	57	45	26	274
Lot II (Stilbestrol)	104	65	76	58	21	324
Lot III (Dienestrol)	99	60	73	56	25	313
Lot IV (Hexestrol)	107	65	64	62	28	326

[1] 11-day period.

animals. The majority of this increased utilization of feed was in the form of ground corn cobs and Supplement A since all four lots consumed approximately the same amount of these feeds daily, whereas the hormone-fed steers consumed more corn than the control steers. This improvement in feed efficiency may be due to increased cellulose and nitrogen utilization as suggested by the artificial rumen studies of Brooks *et al.* (1954).

Effect of Oral Hormones on External Appearance

As reported by Andrews *et al.* (1954) the subcutaneous administration of estrogen pellets to cattle is usually accompanied by recognizable pelvic, mammary and behavior changes. Photographic records were made of the increase in teat length caused by feeding female-like hormones. The onset, degree of manifestation and duration varies with dosage, time and individual animals. In the present experiments there was an increase in teat length, some elevation of the tail head and a relaxation of the lumbar vertebrae. These physiological changes were not as marked as those produced in previous research by implantation of steers with

estrogenic pellets (Andrews *et al.* 1954). There was a suggestion that the effects were more pronounced in the stilbestrol and hexestrol-treated lots.

Effect of Oral Hormones on Carcass Grade

Official federal carcass grades indicated that stilbestrol-fed steers produced carcasses that were slightly inferior to those from either the dienestrol or hexestrol-fed lots, and especially from the control lot which received no hormone (table 3). Similar effects were reported by Andrews

TABLE 3. EFFECT OF ORALLY ADMINISTERED HORMONES ON FEDERAL CARCASS GRADE AND DRESSING PERCENTAGE

	Dressing percent	Low Good	Av. Good	High Good	Low Choice	Av. Choice	High Choice
Lot I, control	58.1	0	1	1	4	2	2
Lot II, stilbestrol	58.2	1	0	3	4	2	0
Lot III, dienestrol	58.4	0	0	0	7	3	0
Lot IV, hexestrol	58.4	0	1	1	3	5	0

et al. (1949) in the case of carcasses from lambs that had been implanted with diethylstilbestrol.

Effect of Oral Hormone in Residue of Meats

Assay of the meat from steers fed stilbestrol, dienestrol or hexestrol at levels of 10 mg. of hormone per steer per day did not reveal any significant estrogenic activity (table 4). This was true whether the hormones were fed up to the day prior to slaughter or whether the hormone-feeding was discontinued one week prior to slaughter.

Summary

The daily oral administration of 10 mg. of either diethylstilbestrol or hexestrol to two-year-old steers on a fattening ration resulted in highly significant increased gains of 50 lb. and 52 lb. per steer, respectively, over the controls receiving no oral hormone, during a 123-day trial.

Steers fed 10 mg. of dienestrol daily gained slightly less (39 lb. more than the controls) than stilbestrol or hexestrol-fed steers, but significantly more (odds 5 percent) than the control steers.

The estrogen treated steers required from 9 to 12 percent less feed per unit gain.

Recognizable pelvic and mammary changes were induced by oral administration of 10 mg. daily per steer of diethylstilbestrol, dienestrol diacetate, or hexestrol.

The carcasses from stilbestrol-fed steers were federally graded as slightly inferior to hexestrol or dienestrol-fed steers or to the control steers which received no hormone.

Assay of the meat from hormone-fed steers showed no residual hormone in the meat. This was true whether the feeding of hormones to the steers was discontinued 1 or 7 days prior to slaughter.

TABLE 4. EFFECT OF MEAT FROM HORMONE-FED STEERS ON THE UTERINE WEIGHT OF CASTRATE FEMALE MICE

Source of Meat Fed	Body wt.	Uterine wt.	% Body wt.
	gm.	mg.	
Control—no hormone	28.0	19.7	.071
Stilbestrol fed (Discontinued 7 days prior to slaughter)	28.3	18.5	.066
Stilbestrol-fed	24.4	16.3	.067
Dienestrol-fed (Discontinued 7 days prior to slaughter)	29.4	19.4	.066
Dienestrol-fed	25.3	17.4	.070
Hexestrol-fed (Discontinued 7 days prior to slaughter)	27.4	18.6	.067
Hexestrol-fed	26.7	15.8	.059

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AUREOMYCIN EFFECTS—GROWTH AND DIGESTIBILITY STUDIES WITH IDENTICAL TWIN CALVES

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AUREOMYCIN has gained widespread use in the rations of young dairy calves. Numerous studies by Bartley *et al.* (1950) (1954), Bloom and Knodt (1953), Jacobson *et al.* (1951), Knodt and Ross (1952) MacKay *et al.* (1952) and Rusoff *et al.* (1951) have shown beneficial effects through increasing growth rates and decreasing the incidence of scours. Although some reports indicate that antibiotics effect an improvement in the efficiency of feed utilization—Bell *et al.* (1951), Murley *et al.* (1952) and Rusoff *et al.* (1953)—others have recorded no difference in the amount of feed required to produce a unit of gain in body weight—Bartley *et al.* (1950), Bell *et al.* (1951), Jacobson *et al.* (1951), Loosli *et al.* (1951), Murley *et al.* (1952) and Rusoff *et al.* (1953). Murley *et al.* (1952) found no difference in the utilization of dry matter, carbohydrates, nitrogen, or ash between the control and aureomycin-fed calves. The calves were fed a restricted diet of non-fat dry milk solids, and aureomycin did not cause an increase in rate of gain. Bell *et al.* (1951) obtained a marked decrease in the digestibility of all nutrients of diets containing aureomycin when fed to steers. However, no work has been done to study the digestibility of a standard calf ration by young calves in which a growth response to aureomycin has been obtained.

Experimental Procedure

Four pairs of identical twin male calves purchased from farmer herds at about one week of age were used in this trial. The diagnosis of zygosity was based on the morphological traits of the calves, as hair color, skin pigmentation, pattern of white markings, hair whorls, nose prints, head and body conformation. All calves were apparently healthy when brought to the Experiment Station. They were placed on trial at once and continued on experiment up to 8 weeks of age.

One calf (A) of each pair served as the control. The second calf (B) was fed a soluble form of aureomycin in the milk at the daily rate of 30 mg. per 100 lb. of body weight.

Vermont Agricultural Experiment Station, Journal Series No. 48.

The calves were housed in individual pens in the main barn which is separate from the regular Experiment Station calf barn. They were fed whole milk for the first month and skim milk until the end of the trial. A commercial concentrate which contained no antibiotic supplement was fed up to a maximum of four lb. daily. Hay was fed *ad libitum*.

At the end of an 8-week growth period, the calves were placed in digestion crates and carried for a 7-day collection period. The calves in pairs I and II were allowed feed, free access, while the calves in pairs III and IV were restricted in feed intake so that each calf in a pair consumed the same amount of feed as its twin.

Feed and feces analyses were made according to the methods of A.O.A.C. (1950).

TABLE 1. GROWTH AND FEED UTILIZATION DATA ON IDENTICAL TWIN CALVES DURING 8-WEEK PERIOD

Calf No.	Breed	Initial Weight	Final Weight	Total Gain	Average Daily Gain	T.D.N. Intake	T.D.N. per Pound of Gain
		lb.	lb.	lb.	lb.	lb.	lb.
				Control			
I-A	Hol.	68	149	81	1.45	116.2	1.44
II-A	Ayr.	70	160	90	1.61	137.0	1.52
III-A	Jer.	46	110	64	1.14	104.2	1.63
IV-A	Guer.	71	138	67	1.20	113.5	1.69
Av.		63.8	139.2	75.5	1.35	117.7	1.57
				Aureomycin Supplemented			
I-B	Hol.	68	154	86	1.54	116.8	1.36
II-B	Ayr.	71	171	100	1.78	143.9	1.44
III-B	Jer.	46	120	74	1.32	112.3	1.52
IV-B	Guer.	71	147	76	1.36	119.0	1.56
Av.		64.0	148.0	84.0	1.50	123.0	1.47

Results and Discussion

The growth and feed consumption data over the 8-week growth period are presented in table 1. The average daily gain for the control calves was 1.35 lb. as compared to 1.50 lb. for the calves receiving aureomycin. The difference was significant (P less than 0.01).

The aureomycin-fed calves consumed only slightly more T.D.N. than the control calves during the 8-week growth period. The amount of T.D.N. required per pound of gain for the control calves was 1.57 while

that for the aureomycin-fed calves was 1.47. It was significantly higher for the control calves, (P less than 0.01).

The digestive coefficients for dry matter, ash, protein, crude fiber, nitrogen-free extract and fat as obtained for each calf are presented in table 2. There were no differences in these coefficients which could be attributed to aureomycin feeding. Considerable variation was noted in the crude fiber values, but this variation occurred with both the control and the supplemented calves.

TABLE 2. DIGESTION COEFFICIENTS OF A STANDARD RATION FOR DAIRY CALVES, WITH AND WITHOUT AUREOMYCIN SUPPLEMENTATION

Calf No.	Dry Matter	Ash	Protein	Crude Fiber	Nitrogen-Free Extract	Fat
	%	%	%	%	%	%
			Control			
I-A	79.9	73.5	80.3	48.0	85.5	80.8
II-A	84.0	75.9	85.1	50.5	88.3	85.0
III-A	79.3	66.0	77.2	46.7	85.4	72.7
IV-A	76.0	70.9	76.5	33.9	82.0	78.7
Av.	79.8	71.6	79.8	44.8	85.3	79.3
			Aureomycin Supplemented			
I-B	81.3	75.8	84.3	44.7	86.0	83.8
II-B	79.6	70.8	78.3	33.8	85.6	82.1
III-B	78.6	66.2	76.7	44.7	84.2	80.4
IV-B	77.1	68.4	77.9	39.5	82.7	80.2
Av.	79.2	70.3	79.3	40.7	84.6	81.6

Summary

Aureomycin increased the growth rate and improved the efficiency of feed utilization of young calves until eight weeks of age. A 7-day collection period at the end of the 8-week growth period showed practically no differences in the digestibility of dry matter, ash, protein, crude fiber, nitrogen-free extract or fat when aureomycin was included in the ration.

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THE EFFECTS OF THE CORN-ALFALFA HAY RATIO ON THE DIGESTIBILITY OF THE DIFFERENT NUTRIENTS BY CATTLE

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CORN and alfalfa hay are two of the commonly used feeds for fattening beef cattle. How the proportion of corn to alfalfa hay affects the utilization of the complete ration has not been thoroughly studied with cattle.

Although there is considerable information available on the feed lot performance of steers fed on rations containing different proportions of concentrates to roughage, there is little information available on the effect of the corn-alfalfa hay ratio on the digestibility of the different nutrients. Dowe *et al.* (1950 and 1951) reported the results of two group feeding trials in which rations containing the following proportions of concentrates to alfalfa hay were fed: 1:1, 2:1, 3:1, 4:1, and 5:1. The steers fed the 3:1 ratio made the largest gains in the 1950 trial while in the 1951 trial the steers fed the 2:1 ratio made the largest gains. Keith *et al.* (1952) individually fed 60 steer calves and 40 yearling steers rations containing the following concentrate to hay ratios: 1:2, 1:1, 2:1, 3:1, 4:1, and 1:3. The steer calves fed the ratio of concentrate to hay of 2:1 made the most rapid average daily gain of 2.01 lb. The yearling steers fed the 3:1 ratio made the largest average daily gains of 1.91 lb. Connell (1952, 1953 and 1954) presented the results of three group feeding trials in which fattening steers were fed rations containing different ratios of concentrates to roughage. In the 1952 and 1954 trials the steers fed the 3:1 ratio made the most rapid gains while in the 1953 trial the steers fed the 2:1 ratio made the largest gains. Richardson, Smith and Cox (1953) group-fed steers on rations containing the following concentrate to hay ratio: 1:1, 3:1, and 5:1. These workers reported that all lots made satisfactory gains, but that the group-fed 3:1 ratio of concentrates to roughage made the largest average daily gains. Stanley (1953) group-fed steers rations containing the following con-

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centrate to hay ratio: 2:1, 1:1, 1:2, and 1:3. Average daily gains made by the steers fed the 4 rations respectively were: 2.71, 2.66, 2.52, and 2.46 lb.

It is of interest to note that in most of the feeding experiments the steers fed the ration containing the highest levels of concentrates did not in all cases produce the largest gains. Generally, the cattle fed rations containing 2 or 3 parts of concentrates to 1 of roughage have made the greatest gains.

The present study was designed to obtain information on the digestibility of mixed rations containing different ratios of concentrates to alfalfa hay.

Procedure

Two series of digestion studies involving a total of 28 digestion trials [2] were conducted to determine the digestibility of the dry matter, crude protein, crude fiber, ether extract, and nitrogen-free extract contained in the 5 ratios: 1:1, 2:1, 3:1, 4:1, and 5:1. The percentage of digestible protein in each of the ratios was calculated using the digestion coefficients published by Morrison (1948). Where needed, soybean oil meal was added to increase the percentage of digestible protein to the level recommended by the National Research Council (1950). Thus, all ratios provided approximately the same level of crude and digestible protein.

Series 1 consisted of 9 digestion trials. One trial was completed with the 5:1 ratio and 2 trials were completed with each of the other 4 ratios. The test animals in Series 1 were yearling bulls, either Hereford or Aberdeen-Angus bulls produced in the college herds.

Series 2 of the digestion trials involved 2 collection periods, A and B, and a total of 19 digestion trials. During period A, 9 trials were completed. One trial was completed with the 4:1 ratio and 2 trials with each of the other 4 ratios. During period B, 10 trials were conducted, 2 trials with each of the 5 ratios. The test animals used in Series 2 were grade Hereford steer calves.

During the digestion trials the test animals were individually fed twice daily. They were allowed as much time as needed to consume the ration offered. The chemical composition of the feeds used is given in table 1.

During the trials the test animals were allowed to become accustomed to the rations and routine of the digestion trials before the precollection

[2] Trial refers to 1 animal fed 1 ration.

periods were started. Precollection periods were of 10 days duration followed by collection periods also of 10 days duration.

The feces were collected by means of a harness and canvas sack designed by Garrigus and Rusk (1939). Feces were emptied from the bags twice daily. After weighing and thorough mixing of the feces voided a 5 percent sample was taken. The fecal samples were preserved with thymol in one gallon glass jars.

TABLE 1. CHEMICAL COMPOSITION OF FEEDS FED IN THE DIGESTION TRIALS

Series	Dry Matter	Ether Extract	Crude Protein	Crude Fiber	N-Free Extract	Ash
	%	%	%	%	%	%
			Corn			
1	88.8	3.9	8.8	2.0	72.7	1.4
2	89.0	4.6	7.9	2.8	72.3	1.4
			Alfalfa Hay			
1	92.1	2.0	15.4	29.1	37.0	8.6
2	89.7	2.3	14.7	30.6	34.3	7.8
			Soybean Oil Meal			
1	92.5	4.8	43.5	5.3	32.8	6.1
2	91.7	1.3	45.8	7.8	30.9	5.9

Feed samples were taken daily throughout the duration of the trials. Proximate chemical analyses (A.O.A.C. 1945) were made at the close of each collection period on composite aliquot samples of feeds and of feces. Fecal nitrogen was determined before the fecal samples were dried.

Since it was desired that within each series of digestion trials all animals consume equal amounts of feed, the quantity of the least palatable ration was used as the basis for feeding all animals. Preliminary check periods were used to determine the amount of each ration that would be consumed. During these check periods it was noted that the animals receiving the 4:1 and 5:1 ratios were erratic in consuming their feed rations, and that they would selectively consume the hay and refuse the concentrates. The maximum amount of feed that the animals receiving the 4:1 and 5:1 ratios would consume without weighback was determined and used as the level of feeding for all animals within a series.

Results and Discussion

The results of the digestion trials are presented in tables 2, 3, 4, and 5.

TABLE 2. TOTAL FEED CONSUMED AND FECES VOIDED IN DIGESTION TRIALS

Series	Period	Ratio of Concentrates to Roughage	Animal Number	Feed Consumed				Feces Voided	
				Corn	Alfalfa	Soybean	Total	Wet	Dry
				gm.	gm.	gm.	gm.	gm.	gm.
1		1:1	53	33980	33980	none	67960	78069	17331
1		1:1	69	33980	33980	none	67960	93582	20775
2	A	1:1	84	22680	22680	none	45360	58486	12341
2	B	1:1	96	22680	22680	none	45360	58720	13740
2	B	1:1	270	22680	22680	none	45360	52555	12718
2	A	1:1	406	22680	22680	none	45360	67158	13902
1		2:1	24	45300	22660	none	67960	89380	21183
1		2:1	39	45300	22660	none	67960	87705	20260
2	B	2:1	84	30200	15160	none	45360	58891	13898
2	A	2:1	96	30200	15160	none	45360	54792	14027
2	A	2:1	270	30200	15160	none	45360	44341	10376
2	B	2:1	406	30200	15160	none	45360	68343	15172
1		3:1	67	50980	16980	none	67960	64467	16117
1		3:1	76	50980	16980	none	67960	76752	18651
2	A	3:1	49[1]	27216	9072	none	36288	29117	7920
2	A	3:1	68	34020	11340	none	45360	49483	11480
2	B	3:1	93	34020	11340	none	45360	52615	13996
2	B	3:1	470	34020	11340	none	45360	53163	14248
1		4:1	62[1]	43504	10864	1264	55632	40000	10760
1		4:1	70[1]	43504	10364	1264	55632	36291	9436
2	B	4:1	49	36280	9030	1060	46420	49867	13913
2	B	4:1	68	36280	9080	1060	46420	58358	13189
2	A	4:1	79	36280	9080	1060	46420	52517	13864
1		5:1	17	45312	8912	2544	56768	47100	13000
2	B	5:1	64	37580	7780	2120	47480	39204	9409
2	B	5:1	79	37580	7780	2120	47480	61480	16642
2	A	5:1	93[1]	30064	6224	1696	39984	28429	7790
2	A	5:1	470	37580	7780	2120	47480	44601	12667

[1] 8-day collections.

TABLE 3. CHEMICAL COMPOSITION OF FECES [1]

Series	Period	Ratio of Concentrates to Roughage	Animal Number	Composition (%)					
				Dry Matter	Ether Extract	Crude Fiber	Crude Protein	N-Free Extract	Ash
1		1:1	53	22.2	1.4	7.6	3.5	8.0	1.7
1		1:1	69	22.2	1.1	7.2	3.4	8.9	1.6
2	A	1:1	84	21.1	0.69	8.8	3.3	6.9	1.4
2	B	1:1	96	23.4	0.74	8.8	3.2	10.7	1.4
2	B	1:1	270	24.2	0.86	10.1	3.3	9.9	1.1
2	A	1:1	406	20.7	0.56	9.1	3.8	5.9	1.3
		Average		23.3	0.89	8.6	3.4	8.4	1.4
1		2:1	24	23.7	1.1	5.8	3.3	10.6	2.9
1		2:1	39	23.1	1.0	6.9	3.2	10.7	1.3
2	B	2:1	84	23.6	0.71	8.1	3.2	10.4	1.2
2	A	2:1	96	25.6	0.59	7.6	3.8	12.4	1.2
2	A	2:1	270	23.4	0.68	9.8	3.9	7.6	1.4
2	B	2:1	406	22.2	0.80	6.8	2.7	10.7	1.2
		Average		23.6	0.81	7.5	3.4	10.4	1.5
1		3:1	67	25.0	1.7	6.1	4.2	11.7	1.3
1		3:1	76	24.3	1.2	5.6	3.8	12.3	1.4
2	A	3:1	49 [2]	27.2	0.81	7.3	4.4	13.2	1.5
2	A	3:1	68	23.2	0.66	6.9	3.7	10.7	1.2
2	B	3:1	93	26.6	0.83	6.8	3.9	15.1	1.2
2	B	3.1	470	26.8	0.81	6.3	3.6	14.4	1.7
		Average		25.5	1.00	6.5	3.9	12.9	1.4
1		4:1	62 [2]	26.9	1.5	4 7	4.8	14.6	1.3
1		4:1	70 [2]	26.0	1 9	5.1	5.0	12.5	1.5
2	B	4:1	49	27.9	0.99	7.1	4.2	14.2	1.4
2	B	4:1	68	22.6	0.82	5.9	3.4	11.2	1.3
2	A	4:1	79	26.4	0.81	6.4	4.1	14.0	1.1
		Average		26.0	1.20	5.8	4.3	13.3	1.3
1		5:1	17	27.6	1.3	4.1	4.4	16.3	1.5
2	B	5:1	64	24.0	1.0	6.2	4.9	11.9	1.4
2	B	5:1	79	27.1	0.83	4.7	3.7	16.6	1.3
2	A	5:1	93 [2]	27.4	0.68	7.3	4.7	13.4	1.3
2	A	5:1	470	28.4	0.72	6.3	4.6	15.2	1.6
		Average		26.9	0.91	5.7	4.5	14.7	1.4

[1] Analyses by C. R. Anderson, Assistant Agricultural Chemist.
[2] 8-day collections.

TABLE 4. COEFFICIENTS OF APPARENT DIGESTIBILITY OF RATIONS

Series	Period	Ratio of Concentrates to Roughage	Animal Number	Dry Matter	Ether Extract	Crude Fiber	Crude Protein	N-Free Extract	TDN
				%	%	%	%	%	%
1		1:1	53	71.8	45.5	43.9	66.8	83.2	63.6
1		1:1	69	66.2	48.6	36.2	61.3	77.7	58.9
2	A	1:1	84	69.6	74.2	32.1	62.3	83.3	62.5
2	B	1:1	96	66.1	72.2	31.8	63.5	74.1	57.6
2	B	1:1	270	68.6	71.1	29.9	66.1	78.4	59.8
2	A	1:1	406	65.7	76.0	19.3	50.2	83.5	59.3
	Average			68.0	64.6	32.2	61.7	80.0	60.3
1		2:1	24	65.4	55.7	30.9	69.7	77.1	64.4
1		2:1	39	66.8	60.5	19.3	71.2	72.3	63.8
2	B	2:1	84	65.7	75.9	13.0	59.3	77.3	60.2
2	A	2:1	96	65.3	81.4	24.1	54.9	74.8	60.1
2	A	2:1	270	74.4	82.6	20.8	62.6	87.5	68.1
2	B	2:1	406	62.5	68.5	15.3	60.2	72.9	57.3
	Average			66.7	70.8	20.6	63.0	77.0	62.3
1		3:1	67	73.5	52.9	34.0	61.9	82.6	66.2
1		3:1	76	69.4	60.4	27.9	58.9	78.2	63.2
2	A	3:1	49[1]	75.5	83.8	39.9	63.2	83.2	69.8
2	A	3:1	68	71.6	82.1	22.8	58.0	87.5	70.1
2	B	3:1	93	65.4	76.1	19.1	53.1	72.1	59.1
2	B	3:1	470	64.8	76.4	24.3	55.8	73.2	60.6
	Average			70.0	72.0	28.0	58.5	79.5	64.8
1		4:1	62[1]	78.4	69.6	54.1	68.3	83.8	71.3
1		4:1	70[1]	81.1	63.7	54.9	70.0	87.4	73.4
2	B	4:1	49	66.4	73.9	8.7	55.4	76.1	61.7
2	B	4:1	68	68.2	74.7	11.4	57.9	78.0	63.5
2	A	4:1	79	66.5	77.5	13.3	54.0	75.2	61.8
	Average			72.1	71.9	28.5	61.1	80.1	66.3
1		5:1	17	74.4	70.0	46.9	68.0	79.3	68.3
2	B	5:1	64	77.8	79.7	32.4	62.4	84.7	70.8
2	B	5:1	79	60.7	73.7	19.8	55.4	66.6	57.0
2	A	5:1	93[1]	77.0	87.5	27.9	67.1	84.4	71.5
2	A	5:1	470	70.1	64.0	21.9	59.6	77.8	63.9
	Average			72.0	75.0	29.8	62.5	78.6	66.3

[1] 8-day collections.

On the third day of the collection period of Series I, one of the 2 bulls assigned to the 5:1 ratio refused his feed and was removed from the trial. On the ninth day of the collection period the two bulls assigned to the 4:1 ratio and the second of the 2 bulls which had been assigned to the 5:1 ratio started fighting and half of the day's collection was lost for the 3 animals. The first 8 days of the collection period was used for these animals.

On the fifth day of Period A of Series II one of the steers assigned to the 4:1 ratio refused his feed and was removed from the trial. On the tenth day steer 93 assigned to the 5:1 ratio and steer 49 assigned to the 3:1 ratio refused their feed, therefore, only the first 8 day's data were used for these animals.

TABLE 5. ANALYSIS OF VARIANCE FOR THE COEFFICIENTS OF APPARENT DIGESTIBILITY OF THE NUTRIENTS STUDIED

Source	D/f	Dry Matter	Crude Protein	Crude Fiber	N-Free Extract	Ether Extract	TDN
Series	1	69.3	305.3*	1595	18.2	1941**	55.7
Rations	4	32.0	14.5	113	9.7	83	39.3
Rations x series	4	35.5	33.9	325**	25.7	71	20.5
Error	18	21.6	18.9	37	34.3	31	18.8

* $P \leq .05$.
** $P \leq .01$.

Staples and Dinusson (1951) presented data comparing the relative accuracy of seven-day and ten-day collection periods in digestion trials. These workers found that the loss in efficiency for the digestion coefficients of the nutrients studied was less than 2 percent in all cases except that of nitrogen-free extract which showed an efficiency loss of 6.83 percent.

The chemical composition of the feces voided is presented in table 3. These data indicate that the percentages of dry matter, crude protein, and nitrogen-free extract in the feces increased as the concentrates in the rations increased, while the percentage of crude fiber in the feces increased as roughage in the rations increased.

The coefficients of apparent digestibility were calculated in the manner explained by Morrison (1948) and Maynard (1951). Individual and average coefficients of apparent digestibility as determined in these trials are shown in table 4. An analysis of variance was calculated on the apparent digestibility of each of the classes of nutrients studied. The mean squares are given in table 5.

The differences between ratios for average coefficients of apparent digestibility of dry matter were not large but did show a tendency to increase as the level of concentrates in the rations was increased. From the highest to the lowest average coefficient for dry matter the ratios ranked, 4:1, 5:1, 3:1, 1:1, and 2:1.

The average coefficients of apparent digestibility for crude protein did not show any striking differences between ratios. In fact the pattern was rather inconsistent. When the average coefficients were arranged in order from the highest to the lowest they were: 62.96, 62.50, 61.69, 61.13, and 58.48 percent for the 2:1, 5:1, 1:1, 4:1, and 3:1 ratios respectively. Wide differences between ratios were not expected, since the apparent digestibility of the crude protein in corn and alfalfa hay is approximately equal and the rations contained approximately the same percentage of digestible protein.

The average digestion coefficients for ether extract tended to increase as the level of concentrates in the rations was increased. The increase in apparent digestibility between the 1:1 ratio and the 2:1 ratio was approximately 14 percent, between the 2:1 ratio and the 3:1 ratio it was approximately 1 percent. The apparent digestibility of ether extract in the 3:1 ratio and in the 4:1 ratio was approximately equal. The increase in apparent digestibility of ether extract between the 4:1 ratio and the 5:1 ratio was approximately 3 percent. The differences in apparent digestibility of ether extract between ratios became less as the level of concentrate was increased in the rations.

Carbohydrates are determined as two groups in the routine analysis of feed stuffs, they are: (1) crude fiber and (2) nitrogen-free extract.

The average coefficient of apparent digestibility for crude fiber was highest in the 1:1 ratio, lowest in the 2:1 ratio, and approximately equal in the other ratios.

The coefficients of digestibility for nitrogen-free extract were similar for all ratios. Maynard (1951) stated, "since the figure is determined by difference instead of directly, it includes the cumulative errors of the other determinations and thus is not an exact value." Generally, the nitrogen-free extract provides the major portion of the total digestible nutrients in feeds.

The percentages of total digestible nutrients increased in the rations when calculated using the coefficients of apparent digestibility obtained in these trials. Since grains and roughages do contain different percentages of total digestible nutrients, it is logical to expect rations which contain different proportions of concentrates and roughages to differ in

total digestible nutrient contents. The lack of significance observed here may have been due to insufficient data.

When the coefficients of apparent digestibility for the nutrients studied were analyzed statistically the differences in apparent digestibility of the nutrients between ratios were not significant. Significant differences in average coefficients of apparent digestibility between the two series of trials were observed for crude protein, ether extract, and crude fiber. These trials were not designed to study the comparative ability of bulls and steers to utilize nutrients, but the results do raise the question as to whether or not bulls and steers differ in their abilities to digest specific nutrients.

Summary

A total of 28 digestion trials were carried out to study the apparent digestibility of the dry matter, crude protein, crude fiber, ether extract and nitrogen-free extract of rations containing the following ratios of corn to alfalfa hay: 1:1, 2:1, 3:1, 4:1, and 5:1.

The dry matter, crude protein, and nitrogen-free extract in the feces increased as the corn in the rations was increased. The apparent digestibility of the dry matter and the ether extract increased as the corn in the rations was increased.

The coefficients of apparent digestibility for nitrogen-free extract, crude fiber, and protein were similar for all rations.

The rations were mixed to provide approximately equal levels of digestible protein. The coefficient of apparent digestibility of the crude protein in corn is similar to the coefficient of apparent digestibility of the crude protein in alfalfa hay, therefore, no wide differences between ratios were expected.

When the coefficients of apparent digestibility for the nutrients studied were analyzed statistically the differences in apparent digestibility of the nutrients between ratios were not significant.

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RESPONSES OF BEEF CATTLE TO HIGH AMBIENT TEMPERATURES [1]

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BEEF cattle taken from temperate climates to tropical and subtropical areas tend to degenerate and become unproductive (Bonsma, 1947, 1949, 1951; Rhoad, 1935; Findlay, 1950). In the southern and southwestern regions of the United States this problem is not as critical as in some other areas of the world, but it is of considerable importance. Cattle from the temperate zones begin to show signs of stress from heat at an air temperature of about 85° F. Temperatures this high or higher have been recorded for all months of the year at the United States Department of Commerce Weather Bureau Station at Waco, Texas (1953) (approximately 25 miles from the site of this study). It has been observed by many workers that breeds indigenous to hot climates are more heat tolerant but of lower producing ability than cattle developed in temperate zones. Because of this, much crossing of cattle adapted to tropical and temperate climates has taken place. There is a need for information which will serve as a guide in improving these crosses and purebreds for producing ability and still maintaining heat tolerance. Phillips (1950) states, in referring to cattle of tropical regions, that "the relative importance of variations in heat tolerance will increase as infectious disease comes under control, pastures are improved, and innate potentialities for production are increased by other means."

The purposes of this study were (1) to determine the variation in heat tolerance among a relatively large number of cattle, (2) to evaluate the accuracy of heat chamber data indicative of responses under natural conditions, and (3) to determine the use which might be made of such data in selecting beef cattle superior in economic characteristics.

Materials

This study was conducted at Bluebonnet Farm, McGregor, Texas as part of a beef cattle breeding project designed to produce cattle superior in rate and efficiency of gain, carcass value, reproductive efficiency and

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[3] Bluebonnet Farm, McGregor, Tex.

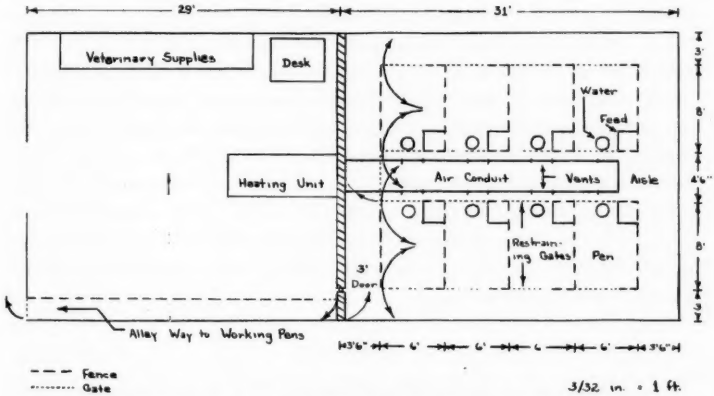

Figure 1. Bluebonnet Farm heat chamber. Floor plan including air conduit suspended from ceiling.

heat tolerance. A total of 366 cattle was studied including bulls, heifers, and steers, principally of the Hereford and Brahman breeds and the cross (F_1) between these breeds. A few Red Polls and Santa Gertrudis were also available. Data were collected from November 1952 until September 1953 from calves dropped in 1951 and 1952.

The heat chamber, which was completed in September 1952, was housed in a building having thick concrete walls on three sides and a thick floor. The roof and front were of frame construction. The details of the arrangements of the interior are shown in figures 1 and 2. The heating unit consists of a forced draft butane heater with an output rating of 180,000 B.T.U. and a continually operating cage type fan

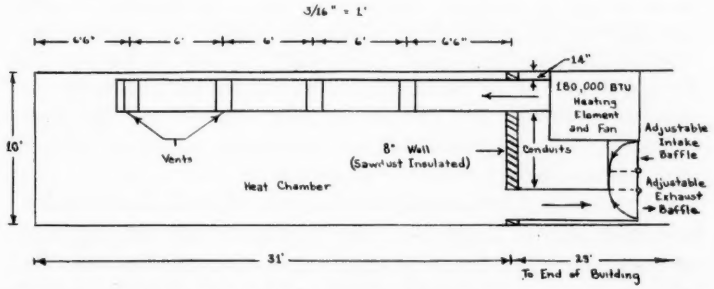

Figure 2. Bluebonnet Farm heat chamber. Cross-section showing heating system.

driven by a ¾ H.P. electric motor. An opening of the hot air duct was placed over the aisle opposite each of eight holding pens. These openings were placed and adjusted so that each holding pen received approximately the same air flow and was maintained at the same temperature. Temperature was thermostatically controlled and fluctuated regularly from 2° F. below the set value of 105° F. to 2° F. above this value. Relative humidity was controlled manually by adjustment of exhaust and input valves and by the addition of moisture to the sanded floor of the aisle in the chamber. With some experience, this method was effective in maintaining the relative humidity within five percent of the set standard of 50 percent.

Methods

Heat tolerance, as measured by physiological responses to atmospheric conditions, was observed both in the heat chamber and under natural field conditions during the summer. All of the cattle observed under field conditions were also observed at least twice in the heat chamber. Animals were penned in the heat chamber the morning before the temperature was to be increased. They were offered feed and water free choice but were limited to a maximum of ten lb. of a ground mixture of hay, grain, and cottonseed meal. During the first day, weight, heart girth, and withers height measurements were taken. Also, subjective scores were recorded for color, temperament, skin area and degree of finish. Other information such as age, sire, dam, etc. were recorded. None of the cattle was halter broken but restraining was accomplished by constructing the chamber holding pens so that gates from both ends could be closed simultaneously on an animal and fastened to hold it in an area about 24 inches in width (see figures 1 and 2). So that the subjects would become accustomed to the surroundings and procedure of observation, they were placed behind the restraining gates. Their rectal temperature was taken several times during the afternoon of the first day. At eight o'clock on the morning of the second day the temperature was increased to 105° F., and held at this level within 2° for eight hours. During the 8-hour exposure, rectal temperature, respiration rate and pulse rate were taken initially and every hour.

Rectal temperature was taken by means of standard mercurial veterinary thermometers. Respiration rate observations were made by counting characteristic respiratory flank movements for a period of time. Time was measured on a stop watch starting at the first count and stopping

when a total of 20 to 30 uninterrupted movements had been counted. Pulse rate was taken manually by palpation of a caudal artery.

Field observations included respiratory counts taken on days with temperatures 95° F. or higher from yearlings in pastures without shade. All animals were branded on both sides so that identification and observation of flank movements was possible at distances of 50 to 60 feet. The observer was always on horse back while making the counts, and the cattle were not unduly disturbed by his presence. Counting always began at 2 P.M. and was completed in approximately one hour. The cattle available for field observation consisted of 92 yearlings. These cattle still possessed growth potential and were weighed every 28 days. From November to the middle of May they were on a rate-of-gain test in the feed lot and were on pasture alone for the remainder of the year. From June 1st to September 30th they were in pastures lacking shade.

Heat chamber observations were taken on each individual at the beginning of the eight-hour period of exposure and every hour thereafter for the entire period. If the initial observation was apparently normal, the eight subsequent values were averaged for body temperature, respiration rate and pulse rate. Field respiration rates was taken on eight different days and the values averaged for each animal. The above averages were used for analyses of the data. Total gain during the time the cattle were in shadeless pasture was used for analysis of summer gain.

Results

The numbers of observations and averages for the above variables are shown in table 1. Analyses of variance of these data are shown in table 2. Gain in weight is shown graphically in figure 3 for the Hereford, Brahman, and F_1 heifers.

To obtain an indication of the relationship of these responses, coefficients of correlation between members of several pairs of variables were computed. All data available for each pair of variables were used in computation, and in all cases at least Herefords, Brahmans and F_1's were represented. These product-moment correlations were computed on both an overall and within breed or cross and sex basis (table 3).

Data suitable for study were analyzed by analysis of variance methods for average chamber body temperature, average chamber respiration rate, average field respiration rate and gain during the summer in order to isolate sire influences. From these analyses (table 4) estimates of

TABLE 1. BODY TEMPERATURE AND RESPIRATORY AND PULSE RATES IN CHAMBER, RESPIRATORY RATE IN FIELD, AND WEIGHT GAIN DURING SUMMER

Breed or Cross and Sex	Av. Chamber Body Temp.		Av. Chamber Resp. Rate		Av. Chamber Pulse Rate		Av. Field Resp. Rate		Summer Gain	
	n	°F	n	no./min.	n	no./min.	n	no./min.	n	lb.
Hereford (H) bulls	23	103.6	23	121.1	19	69.0	8	86.5	4	—22
Brahman (B) bulls	33	102.2	33	69.4	33	67.3	11	45.5	9	— 5
Santa Gertrudis bulls	1	102.6	1	94.3	1	61.3	1	54.0	1	30
F_1 (HXB) bulls	16	102.7	16	87.3	16	74.5	3	61.1	1	15
3/4B–1/4H bulls	2	102.6	6	93.6	2	73.5	2	51.5	—	—
Hereford heifers	94	103.0	94	102.2	94	67.2	31	95.2	30	8
Brahman heifers	24	102.4	24	62.1	24	65.5	8	47.7	9	40
Santa Gertrudis heifers	6	102.9	6	71.3	6	59.0	4	60.0	—	—
Red Poll heifers	3	103.4	3	109.2	3	63.4	3	94.6	—	—
F_1 (HXB) heifers	145	102.4	145	73.3	145	67.0	39	59.6	39	54
Hereford steers	19	104.5	19	107.4	—	—	—	—	—	—

TABLE 2. ANALYSES OF VARIANCE—ALL DATA

Source of Variation	Av. Chamber Body Temp.		Av. Chamber Resp. Rate		Av. Chamber Pulse Rate		Av. Field Resp. Rate		Summer Gain on Pasture	
	D.F.[1]	M.S.[2]	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Between sexes	2	31.23**	2	5,375.05**	1	2,111.09**	1	2,179.94**	1	69.00
Between breed or cross within sex	8	6.27**	8	12,252.09**	8	143.60*	8	4,500.38**	5	11,104.82**
Error	355	0.54	355	341.88	333	71.60	97	48.80	85	1,036.26

[1] Degrees of freedom.
[2] Mean square.

* Statistically significant at the 0.05 level of probability.
** Statistically significant at the 0.01 level of probability.

heritability were obtained by the intra-sire correlation method, where the "between sires within breed or cross and sex" component was significant. The "sire" component was significant only for summer gain. In this case k_0 (average k) was 11.94, s^2 due to sires was 45.05, r_I was 0.0481 and heritability was estimated to be 19.2 percent, i.e., four times 4.81.

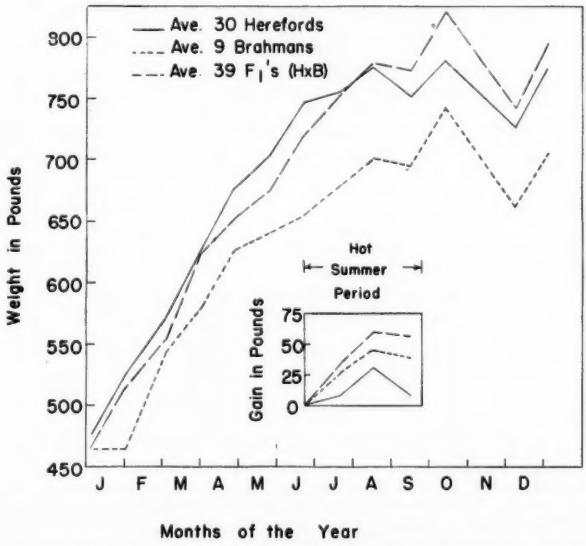

Figure 3. Weights of 78 open, yearling heifers during 1953 and their gains (insert) during the summer period.

For successive heat chamber exposures, repeatability for body temperature was found to be 0.47, 0.62 for respiration rate and 0.32 for pulse rate. Seath and Miller (1948) reported a repeatability of 0.288 for rectal temperature of Holsteins exposed to air temperatures of 80° F. For successive field respiration rate counts it was 0.52.

Discussion

Breed or cross and sex differences were highly significant for chamber body temperature, chamber respiration rate and field respiration rate. Within sexes, a ranking of breeds or crosses according to the degree of heat tolerance as measured by these responses would be approximately

the same as a ranking according to the percentage of Brahman "blood." Similar results have been reported previously by many workers. In the Hereford, Brahman and F_1 groups of the heifers where numbers are larger, it can be seen from the averages that the F_1's are not intermediate but much closer to, or identical with, the more heat tolerant parent, the Brahman. Similar results were found by Rhoad (1938) in working with the Angus as the British breed and by Bonsma (1949) in working with Afrikaners, Shorthorns and Herefords. These differences

TABLE 3. CORRELATION COEFFICIENTS

Character	Type of r	Chamber Body Temperature	Chamber Respiration rate	Field Respiration rate	Winter gain
Chamber	O[1]			.53**	—.09
Body temp.	W[2]			.15**	—.06
Chamber	O	.52**		.59**	.05
Resp. rate	W	.29**		.14**	.06
Summer	O	—.22**	—.23**	—.31**	.02
Gain	W	—.17*	.05	.05	.02
Color[3]	O	—.37**	—.24*	—.46**	
	W	—.15	.21	—.18	
Age in	O	—.32**	—.03		
days	W	—.15**	.44**		
Chamber	O	.06	.48**		
Rel. hum.	W	.12**	.34**		

[1] Over-all.
[2] Within breed or cross and sex.
[3] Subjectively scored on a scale from 0 to 100 so that the numerical value is an estimate of the percentage of all light received by the hair coat which would be reflected in the sunlight.
* Statistically significant at the 0.05 level of probability.
** Statistically significant at the 0.01 level of probability.

suggest that heat tolerance of the Brahman might be at least partially dominant or that some epistasis is present.

The number of heifers among the Herefords, Brahmans and the F_1's was adequate for an analysis of gain during the summer. A significant difference was found between the Herefords and Brahmans and the F_1's were superior to either parental breed as shown in figure 3. Weight losses in all groups occurred during late fall due to short pasture. During the hot summer period, pasture was good for the entire period even though losses occurred in all groups during the latter or hotter portion of this period. After the weather cooled slightly, gains occurred although the pasture was shorter by that time.

The correlation coefficients presented in table 3 give an indication of the relationship between some of the variables studied. The over-all

TABLE 4. ANALYSES OF VARIANCE—DATA SUITABLE FOR HERITABILITY STUDIES

Source of Variation	Av. Chamber Body Temp.		Av. Chamber Resp. Rate		Av. Field Resp. Rate		Summer Gain on Pasture	
	D.F.[1]	M.S.[2]	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Between sexes	2	26.89**	2	4,393.26**	1	2,179.94**	1	69.00
Between breed or cross and sex	4	13.13**	4	19,059.04**	4	9,408.47**	2	25,684.50**
Between sires within breed or cross and sex	47	.21	47	297.48	24	20.33	19	1,677.53**
Error	182	.54	182	301.08	77	33.96	70	890.91

[1] Degrees of freedom.
[2] Mean square.
** Statistically significant at the 0.01 level of probability.

correlations were higher (disregarding sign) than the within-group correlations in cases where there were distinct differences in both variables for the breeds or crosses represented with the exception of winter gain. However, the within group correlations are more informative. Correlations with body temperature are especially important. The coefficient of correlation between chamber body temperature and chamber respiration rate was not large enough to conclude that one of these could be predicted accurately from the other. If both of these responses were closely related to heat tolerance, then heat tolerance could not be assessed with much accuracy from either one taken separately. The same was true for field respiration rate. From an economic standpoint, summer gain is probably the best indicator of heat tolerance studied since it is of concern to the producer because of lowered production during the summer. Gain is not the only measure needed to evaluate production during the summer, but it is one important character and may be closely related to others. If summer gain is assumed to be the best measure of heat tolerance in a beef cattle breeding program with an objective of increasing production, then body temperature would be of little value in predicting gain. Also, respiration rate either in the chamber or field would be of little value.

Color was not significantly correlated with any of the physiological responses studied. If color is important, it would be especially critical in selecting Hereford-Brahman crosses (and perhaps other Brahman crosses), since there is considerable variability in color among animals of this cross. Also, if color is a factor in heat tolerance, it could be more easily demonstrated in cattle with considerable variability than in cattle with little variability. For these reasons coefficients of correlation between color and chamber body temperature, chamber respiration rate and field respiration rate were computed for the F_1 heifers separately since variability in color was greater in this group than in any other. These coefficients were -0.15, 0.38, and -0.29, respectively, and 39 heifers were represented. Although they were not statistically significant, the fact that two of the three increased in absolute value lends support to the belief that color is related to heat tolerance.

Age was significantly correlated with chamber body temperature and with chamber respiration rate. However, the correlation was negative with body temperature and positive with chamber respiration rate. The reason for this difference in relationship is not clear.

Variability in relative humidity was limited in the heat chamber so that the full effect of this climatic factor was not measured. It was sig-

nificantly correlated with chamber body temperature and with chamber respiration rate. The magnitude of the correlation with body temperature was not great enough to be of useful predictive value, but it does indicate a real relationship. Riek and Lee (1948 a, b) found pronounced effects of humidity on body temperature and respiration rate whereas Seath and Miller (1946) did not. The correlation coefficient with respiration rate was greater. This fact indicates that respiratory activity was affected to a greater extent than body temperature. In cattle it appears that at least a major portion of heat loss occurs through evaporative loss in the respiratory passages. Increased humidity would decrease this loss. It may be that a decrease in heat loss caused by increased humidity is compensated for to a certain extent by increased respiration rate. Thus, respiration rate would be expected to be more highly correlated with relative humidity than body temperature. It is believed that relative humidity was a satisfactory measure of humidity for this study since air temperature was maintained within a few degrees of body temperature.

Productiveness during the winter and cooler periods is important because even in the Southwest a majority of the year is included in these periods. Many studies have indicated that productiveness during cooler weather is not an indication of what can be expected during the hotter period. Data from the literature show that breeds adapted to cooler climates do better during cool weather and worse in warm weather than those adapted to warmer climates. This suggests that individuals within a breed might follow the same pattern. To investigate this possibility, gain during the winter was correlated wth body temperature, chamber respiration rate, field respiration rate and summer gain. None of these values was statistically significant on a within breed basis. These relatively low values based on 199 animals consisting of Herefords, Brahmans, and F_1's, indicate that productiveness during the hot season is not physiologically or genetically antagonistic to productiveness during the cool season. Again, however, gain is not the only character that should be considered in determining productiveness.

Pulse rate is not shown in table 3 but correlation coefficients were computed with this variable and all those paired with body temperatures. None of the values was statistically significant. This lack of significance further indicates that this physiological measure is of little use in evaluating heat tolerance.

Heritability estimates computed to determine the degree of effectiveness which could be expected from selection for these characters related to heat tolerance are derived from the analyses of table 4. These sta-

tistics were estimated on a within-breed basis. Statistical significance was lacking between sire groups for all variables except summer gain. In the cases of nonsignificance, heritability was estimated to be zero. Heritability for summer gain was estimated to be approximately 19 percent. Heritability of rate of gain in the feed lot has been estimated to be considerably higher than 19 percent. Patterson *et al.* (1949) have reported estimates close to 100 percent; Patterson *et al.* (1955) reported an estimate of 53 percent; Knapp and Clark (1946 a, b) have reported high estimates and then revised them downward to about 72 percent; and Warwick and Cartwright (1955) have found estimates for Herefords, Brahmans and F_1's between these breeds of 21 percent, 46 percent and 33 percent, respectively and 34 percent for all combined. These estimates are all higher than the estimates found for summer gain. The figures found by Warwick and Cartwright are especially useful in this comparison because the breeds used in both studies are the same (actually, 92 of the individuals are the same) and because the studies were conducted at the same location. Heritability of gain in the feed lot was expected to be higher than for gain on pasture. The estimate reported here is more in line with the value of 12 percent reported by Kincaid *et al.* (1952) which was also computed from data gathered on pasture-fed heifers.

The results of this study indicate that heat tolerance can be increased in British breeds by the infusion of Brahman blood with respect to both physiological responses and rate of gain in the summer. Further, it appears that selection would be effective for rate of gain in the summer within breeds, but that selection based on physiological responses would not be effective in increasing heat tolerance. Under conditions similar to those reported here, field observations are just as effective as heat chamber observations, and summer weight gain is more effective than any other measure. Additional information is needed especially with respect to the relationship between summer performance of mature cattle and summer performance of yearlings as measured by gain in weight while on pasture. More and more, emphasis is being placed on weights of cattle and the use of scales by breeders will probably increase. It is fortunate that a criterion for selecting for heat tolerance appears to fit so well into a program of weighing that is extended through the summer.

Summary

Many reports indicate that production is lowered in cattle during the summer in tropical and subtropical areas and that tropical breeds are

more heat tolerant than exotic breeds. Little work has been reported on the physiological and anatomical differences responsible for varying degrees of adaptability.

In this study a heat chamber was used to subject eight animals simultaneously to temperatures of 105° F. and relative humidity of 50 per cent for an eight-hour period. A total of 366 animal observations were made. Analyses of the data showed significant breed differences with Herefords high, Brahmans low and the F_1 between these intermediate but nearer the Brahman for body temperature and respiration rate. Similar differences were found for respiration rate taken in the field and weight gain during the summer in shadeless pastures except that the order for gain (high to low) was F_1, Brahman and Hereford. Correlations between the above characters and some others that appeared to be related to heat tolerance were mostly significant but of low magnitude.

Summer gain, only, was found to be sufficiently high in heritability (19 percent) to indicate usefulness in selection. Summer gain and winter gain appeared to be negatively correlated on a breed basis but independent within breeds. It appears that summer gain will be more useful in selection for heat tolerance under practical conditions than any other measure studied.

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HERITABILITY OF RATE OF GAIN IN YOUNG GROWING BEEF CATTLE[1]

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IN order to be profitable, beef cattle must have the inherent ability to grow and gain rapidly. The most satisfactory method of studying rate of gain is under the controlled condition of the feed lot. Estimates of heritability of this character which have been reported in the literature are not consistent. Estimates of heritability of rate of gain of beef cattle in the feed lot have been published from the Miles City, Montana station by Knapp and Nordskog (1946a, 1946b) and Knapp and Clark (1947, 1950), from the Balmorhea, Texas station by Patterson *et al.* (1949) and Patterson *et al.* (1954), and from the Virginia station by Kincaid *et al.* (1952). These varied from about 0 to close to 100 percent. The most recent revised estimates reported were: Miles City, 65 percent by the half-sib correlation method and 77 percent by the regression of offspring on dam method; Balmorhea, 53 percent by the half-sib correlation method; and Virginia, 22 percent using available gain data from both sires and progeny. Heritability of gain of heifers on pasture was estimated by Kincaid *et al.* (1952) as 12 percent and by Cartwright (1954) as 19 percent. The estimates computed from data collected while the cattle were on pasture would be expected to be smaller than those estimates from data collected in the feed lot.

In order to determine the influence of pretest treatment upon the performance of calves on test, Patterson *et al.* (1949) and Patterson *et al.* (1954) have correlated various measurements taken at the beginning and end of a gain test. Included in these observations were initial and final age, condition, and grade or body type. They concluded that the only one of these characters that appreciably affects subsequent gain is initial condition.

Materials and Methods

In 1949–50 the first full scale evaluation test for gain was put into operation with bulls and heifers owned by Blue Bonnet Farm. At that

[1] In cooperation with U. S. Department of Agriculture; and is a part of the S-10, Southern Regional Beef Cattle Breeding Project. M. W. Hazen, Animal Husbandman, U.S.D.A., formerly stationed at Bluebonnet Farm, now Supt. Chinsegut Hill Sanctuary, Brooksville, Florida assisted in this work from 1949 to 1952.

[2] Bluebonnet Farm, McGregor, Tex.

time the test was also made available to private breeders for testing bull calves. In 1950–51 the test was opened to private breeders to test heifers as well as bull calves. Heat tolerance studies and carcass evaluations have more recently been made but will be reported separately.

The data reported here were collected during four gain evaluation tests conducted in 1949–50, 1950–51, 1951–52, and 1952–53. The gain evaluation test has been standardized in so far as possible. The present test begins in November and continues for a period of 140 days after an adjustment period of two weeks. The first three tests were each 154 days. Calves must be dropped between Nov. 1 of the previous year and April 30 of the year of the start of the test. The ration for all breeding cattle was premixed and consisted of cottonseed meal, ground milo, ground hegari fodder, ground Johnson grass hay, and either ground alfalfa leaf meal and/or vitamin A concentrate. The mixture consisted of about 35 percent concentrates during the test feeding period except for a portion of the first year during which time the ration for the bulls was 50 percent concentrate. Steers were fed in two groups, one received the same ration as described for the bulls and heifers, the other group was fed a ration of 66–68 percent concentrates. Other than part of the Herefords and the crossbred cattle raised at Bluebonnet, all tested animals have been registered and in sire progeny groups of 2 or more.

Data were collected during these tests for 853 animals, 587 of which were raised at the station. Since two breeds, one cross, and bulls, heifers and steers have been tested over a period of four years, some difficulties in analysis and evaluation were encountered. In an attempt to overcome some of these difficulties, each animal's record was converted to a gain ratio. The gain ratio was computed for each animal by dividing its total gain on test by the average total gain on test of the same year, breed, sex, and ration group and multiplying by 100. For example Hereford bull "BF 831" gained 375 lb. in the 1952–53 test. The average for Hereford bulls that year was 309 lb. so the gain ratio for "BF 831" is (375/309) 100 or 121. It can readily be seen that an average animal would have a gain ratio of 100. An animal above 100 would be above average, below 100 would be below average. Average daily gains, extremes of daily gain and extremes of gain ratio for the Herefords, Brahmans and F_1's between these breeds are shown in table 1.

Results

The gain ratio has proved useful with the problem of actual selection of individuals because it eliminates differences between averages

due to ration, sex, and year within breeds and is simply and quickly computed. Heritability was estimated for rate of gain as indicated by the gain ratio by two methods.

The first estimate was obtained by the method of the correlation between half-sibs. Because of differences in pretest environment and differences due to genetic variation between cow herds of the different ranches where the animals were raised, estimates of heritability were computed separately for those cattle raised at Bluebonnet Farm (BF) and for those raised elsewhere. Also, the Hereford and Brahman as well as the F_1 (Hereford×Brahman) groups were treated separately. All F_1's were raised at Bluebonnet Farm. The analyses of variance and the estimates obtained for each sub-division are given in table 2.

TABLE 1. AVERAGE DAILY GAINS AND EXTREMES OF DAILY GAIN AND GAIN RATIO OF HEREFORDS, BRAHMAN AND F_1's (HEREFORD × BRAHMAN) REPRESENTED IN THE STUDY

Breed, Sex and Ration	Daily Gain in Pounds				Gain Ratio	
	N	Av.	High	Low	High	Low
Hereford bulls						
Low conc. ration	112	2.2	3.1	1.2	141	52
Brahman bulls						
Low conc. ration	69	1.8	2.5	0.9	148	53
F_1 (H×B) bulls						
Low conc. ration	30	2.3	2.7	1.6	123	73
Hereford heifers						
Low conc. ration	193	1.5	2.2	0.5	150	42
Brahman heifers						
Low conc. ration	55	1.2	1.8	0.6	160	60
F_1 (H×B) heifers						
Low conc. ration	169	1.6	2.3	0.8	153	53
Hereford steers						
Low conc. ration	45	2.0	2.5	1.6	125	80
F_1 (H×B) steers						
Low conc. ration	45	1.6	2.2	0.7	147	47
Hereford steers						
High conc. ration	38	2.1	2.9	1.7	132	82
F_1 (H×B) steers						
High conc. ration	115	2.1	2.8	1.0	129	59

The second method was by the regression of offspring on parent and includes Bluebonnet cattle only. Sixty-eight parents with test records were paired with the average of their offspring (n=147) for this calculation. The regression coefficient was 0.29 and heritability was estimated to be 57 percent. Also available were five additional bulls which had

TABLE 2. ANALYSES OF VARIANCE OF GAIN RATIO AND ESTIMATES OF HERITABILITY

Item	All Animals		BF Animals		All Herefords		BF Herefords		All Brahmans		BF Brahmans		BF F_1's	
Between[1]	94	405**	28	614**	40	360**	7	359*	35	374	10	377	17	669**
Within[2]	758	211	558	217	288	172	181	167	88	259	22	287	341	241
k_0Sires[3]	8.8		19.7		7.5		20.3		3.4		2.9		19.6	
r_{I4}	0.09		0.08		0.13		0.05		0.11		0.10		0.08	
$H(\%)$[5]	38		34		51		21		46		41		33	

[1] Between sire progeny groups, degrees of freedom and mean squares.
[2] Within sire progeny groups, degrees of freedom and mean squares.
[3] Average no. of progeny per sire group (Snedecor, 1946).
[4] Intra-sire correlation between progeny.
[5] Estimate of heritability of gain ratio.
* Statistically significant at the 0.05 level of probability.
** Statistically significant at the 0.01 level of probability

gain test records from another station but sired Bluebonnet tested calves. When this additional information was added (n=291), the estimate was changed slightly to 54 percent.

For comparison, estimates for heritability of rate of gain were also computed from the daily gain data using the actual pounds gained. Total gain was not used because the tests were not all of equal duration of time. These estimates were computed using the intra-class correlation method. They were computed for all of the classifications used with the gain ratio analyses and in addition the steers fed the high concentrate were treated separately. These estimates varied for the different groups from 14 to 175 percent.

There has been some variation among the cattle entering the test with respect to age, weight and condition. It is logically suspected that these variables might affect subsequent gain. The correlation coefficient between initial weight and final gain ratio based on 853 animals was 0.11 which was statistically significant. Between initial age and final gain ratio it was 0.00. Differences in initial weight account for only approximately 1 percent of the variation in subsequent gain, and age apparently has no effect. Cattle entering the test have been in medium flesh with few exceptions. A few animals that were considered to have entered in a fat condition gained poorly. Based on these observations it is believed that animals in such condition generally gain more slowly than they would have if they had not been fed so heavily prior to the test.

Discussion

The estimates of heritability of rate of gain using gain ratio reported here show variability ranging from 21 to 57 percent. The estimates using average daily gain varied much more widely. Different groups or samples of genetic material are expected to vary in the degree of heritability of the same character even under identical conditions, but estimates exceeding 100 percent and such differences as found using actual gain can not be accounted for by differences in genetic material.

Some of the differences were anticipated, and certain procedures of the experimental project were designed to account for them. In every case, with the exception of one group represented by 33 animals, the estimates were lower for the Bluebonnet animals than for the entire group tested. All animals at Bluebonnet are treated as nearly alike as possible except for a three-month breeding season in which the cows and calves are in 14 or 15 different pastures. Each year the cows are reas-

signed to their respective breeding groups at random within the breed or cross of the cows. This procedure tends to reduce pretest environmental influences and differences due to genetic make up of the cow herd which are confounded with sire differences in those cattle entered in the test by cooperators.

The estimates derived from the gain ratio data are not true estimates of heritability of rate of gain but are perhaps more useful. Replacements are most logically selected on this or a similar basis which eliminates year and breed differences. Maximum gain is desired rather than a particular rate of gain. Also, records made by progeny of the same sire can be grouped together although the sexes, years, breeds or crosses and rations may be different thus greatly increasing the usable number per sire progeny group. The estimates obtained from the use of gain ratio data are much lower than those obtained from the actual pounds of gain. There are two apparent reasons for this difference. The first is that they are not identical measures of rate of gain. One is a physical measurement of gain while the other is a measure of gain relative to the magnitude of the average gain of the group. The second is that the gain ratio eliminates differences due to year, breed or cross, sex and ration, whereas this can not be done satisfactorily by analysis of gain in pounds on a within year, sex, breed or cross and ration basis with data such as those reported here. In the analyses using daily gain the interaction "sires $\times$ years" was assumed to be nonsignificant and it appears as a part of the term "between sire progeny groups within years and groups." Patterson *et al.* (1954) reported significant interactions between sires and years which caused estimates to appear about twice as high as the ones in which this interaction was isolated. In data reported in this paper the same sires are not represented in enough consecutive years to allow isolation of this component. Since this interaction can not be evaluated, the estimates based on pounds of gain in this study are not considered valid. They are interesting in that they tend to emphasize further the fact that "sire $\times$ year" interactions are probably important and deserve more attention than has generally been the case.

According to Lush (1940) less bias is incorporated in estimates of heritability by use of the method of regression of offspring on parent than with half-sib correlation methods. The estimate of 54 percent obtained by this method in this study is in close agreement with the revised estimates of the Miles City (Knapp and Clark, 1950) and Balmorhea (Patterson *et al.* 1954) works which were 65 to 77 percent and 53 percent respectively. The half-sib correlation estimates of herita-

bility of gain ratio which are slightly lower, ranging from 21 to 51 percent, are within the range of differences that are expected in such studies. The Virginia work (Kincaid *et al.* 1952) indicates lower heritability of this character.

The evidence in the literature and the findings of this work support the contention that heritability of rate of gain of beef cattle in the feed lot under relatively uniform conditions is high. Also it appears that within the limits imposed upon test animals, an individual's ability is accurately evaluated with respect to gain. Heritability of gain may well be higher than for any other economic character in farm animals but not as high as earlier reports indicated. It appears that in a sample of cattle such as those from which data of this work were gathered, that heritability of rate of gain could be expected to be approximately 50 percent.

Based on the assumption that heritability of rate of gain is at least 50 percent the procedures, rules and regulations of the test at Bluebonnet Farm have been modified to emphasize the record of the individual. "Progeny testing" has been necessary in laying the basis of performance testing but now it is indicated that it should be given an incidental position. Testing individuals rather than sires allows many more bulls to be tested and with the additional advantage of obtaining evaluations just as they reach sexual maturity. The same applies for heifers, especially since progeny testing a dam is not feasible. Estimates of heritability such as those suggested above indicate that individual selection would be effective. Even though heritability is apparently lower for the F_1's, it is still of such magnitude as to indicate that attention should be given to the gain records of parents even in a cross breeding program. Individual selection is again indicated although perhaps not as strongly as with the purebreds.

Differences in gaining ability of beef cattle have been found to be great. Cattle are sold by the pound, and weight at market time is probably more important than any other character. This consideration coupled with the fact that heritability of gain is high, indicates that gaining ability should receive more attention than any other character. This is especially true with respect to type and conformation. The fact that these have received so much attention probably accounts for neglect of selection for growth as indicated by rate of gain. The advantage of rapid gaining ability is obvious to the commercial producer. The purebred breeder should set his sights on animals that will do the best job for the producer of beef rather than on show ring standards. Surely,

education will create a greater demand for breeding animals that have some evidence to indicate that they are inherently high gainers. There is little doubt that economic reward awaits the breeder who has had the foresight to plan his selection on such a sound basis as gaining ability.

Summary

Data collected from four annual beef cattle gain-evaluation tests were analyzed. These data were from 853 head consisting of two breeds, one cross and three sex classifications. Cooperating breeders entered 266 of the calves and the station 587. Within the limits included in these data, initial age and weight have no important influence on subsequent gain on test. Determination of heritability from half-sib correlations using actual gain gave high estimates that are not considered valid because "sire×year" interaction could not be satisfactorily removed. Analyses using gain ratio, a relative measure obtained by dividing an individual's gain by the average of his sex, breed, year and ration group, gave estimates ranging from 33 to 51 percent for different classifications of breed or cross and pretest history. Estimates computed from data from station raised cattle only were smaller than from all cattle in all but one classification. This indicates that pretest ranch differences of cooperator cattle which were confounded with sire differences may bias heritability estimates upward. The most reliable estimate is considered to be that obtained by the method of regression of parent on average offspring using gain ratio. Calculated from 73 pairs including 291 offspring, it was 54 percent. These high heritability estimates add overwhelming support to the conclusion that selection for rate of gain will be effective when the individual's own record is used.

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THE HERITABILITY OF SOME ECONOMIC CHARACTERISTICS OF BEEF CATTLE [1] [2]

C. E. Shelby, R. T. Clark and R. R. Woodward [3] [4]

IN the past much emphasis has been placed on visual conformation in the selection of breeding stock in beef cattle. Previous studies have shown there is little relationship between conformation and most economically important traits. The need for more adequate measures of performance led to the development of Record of Performance procedures. These have stressed various growth and carcass characteristics thought to be of economic importance. In order to evaluate their usefulness as criteria for selection, the genetic and environmental relationships existing between and within these characteristics should be investigated.

The present study is an attempt to clarify these relationships in data collected during ten years (1942–1951) of Record of Performance testing at the U. S. Range Livestock Experiment Station at Miles City, Montana. Heritabilities for a number of growth and carcass characteristics in beef cattle are given in this study.

Source of Data

The data used were collected at the U. S. Range Livestock Experiment Station, Miles City, Montana, in experiments conducted cooperatively by the Agricultural Research Service, U. S. Department of Agriculture, and the Montana Agricultural Experiment Station, Bozeman, Montana. They consist of the records of 635 Hereford steers fed in Record of Performance tests from 1942 to 1951, inclusive. The steers are by 88 sires from 9 inbred lines of different pedigree origin. The inbred lines were established by purchasing related bulls and cows from individual herds in the various western states. Each line was maintained as a closed herd with no outside blood being introduced. Selection within the lines was based largely on the results of production tests with some

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[3] Agricultural Research Service, U. S. Department of Agriculture, U. S. Range Livestock Experiment Station, Miles City, Montana.

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attention to conformation. Inbreeding has been relatively mild within the various lines. The average coefficient of inbreeding of the sires in this study was only .08.

The following characteristics were studied: birth weight (lb.), weaning weight (lb.), final weight in the feedlot (lb.), gain in the feedlot (lb.), efficiency of feed utilization (lb. of gain per 100 lb. of total digestible nutrients), shrink (lb. of final weight minus sales weight), slaughter grade (units equivalent to one-sixth of a grade—P.M.A.—B.A.I.—A.R.A.—chart 101, i.e. prime—2, 4, and 6, choice—8, 10, and 12, good—14, 16, and 18, and commercial—20, 22, and 24), dressing percentage (cold carcass weight divided by final weight), carcass grade (units equivalent to one-sixth of a grade—A.M.A.—B.A.I.—chart 102), color of eye muscle (colors range from 1 to 10 indicating light to dark red color, U.S.D.A. color standard A), area of eye muscle (square inches), and thickness of fat (in mm.—average of three measurements of external fat covering the eye muscle, taken at 25, 50, and 75 percent of the longitudinal axis of eye muscle).

Yearling or two-year-old bulls were placed in breeding herds of 25 to 30 grade cows for a period of 45 days. Cows were selected at random except ages were equalized between groups. The basis for selection of bulls has varied much with breeding plans. An attempt has been made to test bulls from all lines. In general, only the faster gaining, heavier bulls of good conformation were used.

At completion of the breeding season the cows were gathered in one large pasture until their calves were weaned. The cows were turned on fall range. They were moved to winter pasture about the first of January. The cows were fed varying amounts of protein supplement. Hay was provided when weather was severely cold or prevented normal grazing. The cows gained or lost weight depending on the amount of vegetation, the severity of the weather, and the amount of supplement fed. They were moved to a single large pasture where calves were dropped during an 8- to 10-week period from March 25 to June 1. Calves remained on the range with their dams until weaned about October 20.

If available, eight steers were selected at random from each sire group to go into the feedlot. Three to eight steers completed the tests each year. The average yearly range in age when placed on test was 50.9 days. Since 1944, steers have had a preliminary feeding period of about ten days. Initial weights were taken on three successive days at the beginning of the trial. The following ration was fed: 6 parts corn

or barley, 3 parts dried beet pulp, 1 part linseed oil meal, 1 part wheat bran, a Vitamin A concentrate and alfalfa hay.

The steers were started on feed with two to three lb. of concentrate and alfalfa hay fed *ad libitum*. At the conclusion of the trial they were consuming about 17 to 18 lb. of concentrate and about 3 to 4 lb. of hay. The actual amounts fed varied with individual appetites. A mixture of 2 parts salt to 1 part bone meal was fed free choice. Steers were individually fed through 1946, and were group fed thereafter. Concentrate was hand fed twice daily, and roughage once daily. The length of feeding trial varied from 252 to 273 days. Individual weights were taken at the end of each 28-day period. Final weights were taken on three successive days at the completion of the trial.

The steers were marketed at South St. Paul, Minnesota, where carcass data were obtained. They were loaded within 24 hours after the last weight was taken on Wednesday and reached South St. Paul on Saturday or Sunday. The steers had a fill of at least 30 hours. They were individually graded on Monday by a committee of three members. Sale weights were taken in the afternoon of the day on which they were sold. The steers were immediately taken to the packing house and were killed the following day. Hot carcass weights were taken on the killing floor. Carcasses were kept in the cooler for 48 hours before carcass data were obtained.

Each carcass was individually graded by a committee of three members. Color readings of eye muscle (cross section of longissimus dorsi muscle at twelfth rib cut) were made using a standard color disc thirty minutes after carcasses were ribbed. Tracings of eye muscle and external fat at the posterior side of the twelfth rib cut were made on transparent non-absorbent paper. The eye muscle tracings were later measured by a compensating polar planimeter. Thickness of fat was an average of three measurements made perpendicular to the longtitudinal axis of the eye muscle at one-fourth, one-half, and three-fourths of the length of the axis.

Method of Analysis

Heritability in the narrow sense is the fraction of the phenotypic variance which is due to the average effects of the genes of the individual members of the population. Its magnitude is important in choosing the method of selection and the breeding plan, in determining the relative amount of emphasis to be given each trait when selecting breeding stock, in estimating the breeding value of individual animals, and in estimating the rate of progress in a selection program.

The methods used to estimate heritability are based on the fact that related individuals generally resemble each other more closely than unrelated individuals. The present study utilizes the correlation between paternal half-sibs to estimate heritability.

The analysis of variance for unequal subclass numbers as outlined by Snedecor (1946) was used to obtain mean squares. Components of variance were estimated by equating the mathematical expectation of each classification to the mean squares and by solving this system of equations by ordinary computational procedures. The assumptions necessary to use this type of analysis are given by Henderson (1953) in method 1.

The following mathematical model was used to estimate components of variance:

$$y_{ikjm}=\mu+y_i+l_{ij}+s_{ijk}+e_{ijkm}$$

Where μ is an effect common to all observations,

y_i is an effect common to all steers born in the ith year,

l_{ij} is an effect common to all steers born in the ith year in the jth line,

s_{ijk} is an effect common to all steers born in the ith year in the jth line from the kth sire,

e_{ijkm} is an effect common to each individual steer.

The components of variance have the following interpretation if mating is random:

The variance within sire groups (σ^2_e) includes all the random environmental variance, ¾ of the genic variance, all of the dominance variance, and a major part of the epistatic variance. The variance due to sires (σ^2_s) contains ¼ of the genic variance plus a small fraction of the epistatic variance. The variance between lines (σ^2_l) reflects to a large extent differences in the average level of genetic merit, but could conceivably include differences in environment. The variance between years (σ^2_y) results from differences in physical environment, in nutrition, and in average level of genetic merit.

Heritability within year line subgroups can be estimated from the following ratio:

$$H=\frac{4\sigma^2_s}{\sigma^2_e+\sigma^2_s}$$

The major advantage of using paternal half-sib correlation to estimate heritability is that this value contains only the additive plus a small fraction of the epistatic portion of the hereditary variance. The accuracy of this method of estimation depends on the number of de-

grees of freedom available for estimating the differences between sires. It is limited by the fact that errors due to sampling or incorrect estimation of environmental influences are multiplied by 4.

Results

Actual means by years are listed in table 1. Mean squares are given in table 2. Components of variance are shown in table 3.

The following heritabilities and 95 percent confidence limits were obtained: birth weight 72 (60–80), weaning weight 23 (3–41), gain 60 (46–72), efficiency 22[5] (2–40), final weight 84 (77–89), slaughter grade 42 (25–57), shrink 91 (88–94), dressing percentage 73 (62–81), carcass grade 16 (0–35), color of eye muscle 31 (13–48), area of eye muscle 72 (60–80), and thickness of fat 38 (20–53).

The analysis of variance was used to separate the effects of differences between years, between lines within years, and between sires within lines. The effects of differences in age at weaning, and in age and final weight at completion of the feedlot test were removed by covariance.

Years

The fraction of the total variation in the various characteristics attributable to differences between years is given in table 3. The yearly averages for most growth characteristics were extremely variable. This could result from differences in climatic conditions and in feeding and management as well as in the average genetic composition of the herd. Differences between years were statistically highly significant in all cases.

Birth weight was only slightly affected by differences between years. The extremely severe winters in 1949 and 1950 and the small amounts of precipitation in 1949 could account for the low birth weights in 1949 and in 1950. Weaning weights varied greatly between years. Since the total milk production of the cow depends to a large extent on the amount of grass available, gain from birth to weaning varies with the amount of annual rainfall. This is shown by the extremely low weaning weights in 1949.

Gain in the feedlot varied considerably between years. A comparison of the means for the first five years with the last five years shows a difference of 80 lb. in gain in favor of the latter period. This reflects the higher amounts of feed offered during the latter period. Steers were

[5] This estimate was based on data from steers individually fed from 1942 to 1946, inclusive.

TABLE 1. MEANS OF SOME ECONOMIC CHARACTERISTICS BY YEARS

Characteristic	Year of Birth										Total or Average
	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	
Total rainfall (in.)	17.76	13.97	15.01	18.95	12.60	17.80	11.75	16.08	8.79	13.66	14.64
Number of steers	62	45	46	53	63	98	85	88	50	45	635
Number of sires	8	6	6	7	8	15	11	13	7	7	88
Birth weight (lb.)	81.9	83.4	79.2	80.2	79.9	82.8	83.9	81.7	74.9	74.8	80.8
Weaning weight (lb.)	415.6	401.7	391.0	373.6	373.3	402.3	419.2	433.8	319.2	393.8	396.9
Weaning age (days)	178.4	174.8	174.6	175.1	174.5	177.4	183.0	188.9	187.7	183.2	180.2
Final weight (lb.)	919.3	923.3	850.7	866.8	864.8	958.9	945.7	966.2	916.9	1017.2	927.7
Days in feedlot	252	273	259	252	252	252	252	252	252	252	254
Gain (lb.)	504.5	528.5	464.5	494.4	483.8	574.2	548.2	549.6	593.0	619.2	538.3
Efficiency (lb.)	19.39	18.40	18.17	19.94	19.25	18.78	18.00	18.01	19.47	18.83	18.76
Shrink (lb.)	30.4	24.0	22.9	39.7	46.6	31.3	26.9	43.7	33.0	60.3	35.6
Slaughter grade (units)	15.7	13.1	15.0	14.4	14.4	14.0	14.6	15.2	17.2	14.8	14.8
Dressing percentage	58.4	59.0	58.0	56.6	56.9	58.3	58.7	57.6	59.3	59.5	58.2
Carcass grade (units)	16.2	13.8	15.2	14.5	14.8	12.4	15.0	17.1	16.2	13.1	14.8
Color of eye muscle	4.5	3.9	2.8	5.5	5.1	4.2	5.5	4.4	5.8	5.7	4.7
Area of eye muscle (sq. in.)	12.36	11.45	9.42	9.48	10.30	10.70	11.57	10.74	10.63	10.60	10.79
Thickness of fat (mm.)	9.4	11.7	11.8	11.5	10.8	15.8	17.4	15.6	17.4	21.0	14.4

TABLE 2. ANALYSES OF VARIANCE FOR SOME ECONOMIC CHARACTERISTICS OF BEEF CATTLE

Item	Mean Squares					
	Total	Between Years	Between Lines Within Years	Between Sires Within Lines	Within Sires	Standard Deviation
Degrees of freedom	634	9	19	59	547	
Birth Weight	71	582**	88	140**	55	7.4
Weaning weight	2952	61679**	2589	3913**	1895	40.7[3]
Final weight	7227	149245**	13624	12575**	4092	63.1[4]
Gain	4721	133801**	8347	5437**	2393	48.9
Efficiency	1.44[1]	28.88**	4.89*	2.65*	1.71[2]	1.31
Shrink	445	7275**	733	831**	279	16.4[5]
Slaughter grade	6.3	63.8**	13.6	8.8**	4.8	2.2
Dressing percentage	2.4	51.3**	3.5	3.8**	1.5	1.2
Carcass grade	8.4	162.1**	11.8	7.6	5.9	2.4
Color of eye muscle	1.5	48.0**	1.5	1.2**	0.7	0.8
Area of eye muscle	1.61	45.66**	1.59	2.01**	0.82	0.81[5]
Thickness of fat	22.3	771.3**	27.4	16.8**	10.4	3.0[5]

[1] This value would be 2.28 if efficiencies had been computed on an individual basis for the last 5 years.
[2] Based on 234 degrees of freedom.
[3] Adjusted by analysis of covariance for age at weaning.
[4] Adjusted for age at the completion of the feedlot period.
[5] Adjusted for final weight at the completion of feedlot period.
* Probability of 0.05.
** Probability of 0.01.

individually fed during the first five years of the experiment and were group fed during the last five years. This change was effected on the suggestion of meat specialists in order to provide the fullest expression

TABLE 3. COMPONENTS OF VARIANCE FROM THE ANALYSES OF VARIANCE IN TABLE 2

Source of Variation	σ^2_e	σ^2_s	σ^2_l	σ^2_y	$\sigma^2_e+\sigma^2_s+\sigma^2_l+\sigma^2_y$
Birth[1]	54.69	11.91	— 3.48	8.84	71.96
weight	76.0	16.5	— 4.8	12.3	100
Weaning[2]	1656.56	100.05	— 14.90	928.91	2670.62
weight	62.0	3.8	— 0.06	34.8	100
Final[3]	3983.38	1058.27	39.37	1284.76	6365.78
weight	62.6	16.6	0.6	20.2	100
Gain	2393.48	425.74	183.33	1941.25	4943.80
	48.4	8.6	3.7	39.3	100
Efficiency[3]	1.6645	0.0958	0.1428	0.2816	2.1847
	76.2	4.4	6.5	12.9	100
Shrink[4]	268.17	79.40	— 4.37	92.4	436.14
	61.5	18.2	— 1.0	21.3	100
Slaughter	4.7952	0.5645	0.3039	0.7085	6.3721
grade	75.2	8.9	4.8	11.1	100
Dressing	1.4637	0.3249	— 0.0199	0.7644	2.5331
percentage	57.8	12.8	— 0.8	30.2	100
Carcass	5.8592	0.2497	0.2649	2.3135	8.6873
grade	67.5	2.9	3.0	26.6	100
Color of	0.7294	0.0616	0.0190	0.7343	1.5443
eye muscle	47.2	4.0	1.2	47.6	100
Area of[4]	0.6593	0.1434	— 0.0218	0.5928	1.3737
eye muscle	48.0	10.4	— 1.6	43.2	100
Thickness[4]	9.3011	0.9687	0.0498	4.8974	15.2170
of fat	61.1	6.4	0.3	32.2	100

[1] The upper row for each characteristic gives absolute values, while the lower row gives percentages of the total variance.
[2] Adjusted by the analysis of covariance for age at weaning.
[3] Adjusted for age of animal at end of feedlot period.
[4] Adjusted for final weight at end of feedlot period.

of carcass potential of the cattle. Final weight composed of both weaning weight and gain shows the same effect but to a lesser extent.

Efficiency and slaughter grade have varied only slightly during the experiment. Differences in both slaughter and carcass grade between years are confounded with differences between committees. The committees scoring the steers have varied between years. One of the three

men on each committee has remained the same each year of the experiment. Any changes in the ideals of a given judge over a period of time would be included in this effect.

Considerable yearly variation in shrink has occurred. This may be attributed in part to differences in final weight, in degree of finish, in handling of the steers enroute to market, and in the amount of fill at St. Paul. Although yearly differences in dressing percentage were slight, they accounted for a relatively large fraction of the total variation. Since individual variation was slight in these data, minor yearly differences may have been unduly emphasized.

Carcass characteristics varied considerably between years. No apparent trends were noticeable with the exception that the average thickness of fat in the last five years was almost double that of the first period. This suggests that a much greater proportion of the increased gain in the last five years was deposited in the form of fat.

The fact that relatively large yearly differences exist for most production characteristics should be considered when selecting breeding stock. Some idea of the relative superiority of the animals within a year can be had by measuring the actual deviations from herd average. Such deviations are not exactly comparable between years if variability changes markedly.

Lines Within Years

Only a small fraction of the total variation in the characteristics studied was due to differences between lines within years. These differences were not statistically significant. Since bulls from the various lines were mated to randomly selected grade cows, line differences in this study are not a true measure of the differences existing between lines in the population. In addition to the differences in line of sire, they may include any differences in feeding and management of the various breeding herds or test lots in a given year.

Of the 88 sires in this study, 57 are from line 1 and 10 are from line 2, while the remaining 21 are from 7 other lines acquired in recent years. Since the females in the grade herd are replaced from within the herd, they have become closely related to line 1.

In several instances the variance components were negative. In these cases, the mean squares for sires within lines were larger than the mean squares for lines within years. This results when the average production of the various sire groups within lines varies considerably, while the

average production of the various lines varies only slightly. The number of degrees of freedom available for comparison between lines within years is few, while the number for sires within lines is relatively large for line 1, but is few for the other lines.

Sires Within Lines

Differences between sires within lines were statistically significant for all characteristics. Since the degrees of freedom available for estimating differences between sires are to a large extent from line 1, the components of variance for sires may be too small if the effects of selection or of inbreeding have tended to reduce the genetic variability in line 1. As selection for certain growth characteristics in line 1 has been practiced for a period of 15 years, it seems logical to assume that the genetic variability for the most highly heritable characteristics has decreased slightly. The average inbreeding of the sires in this study was .08.

A number of estimates of the heritability of economic characteristics in beef cattle have been made at the U. S. Range Livestock Experiment Station at Miles City, Montana. These have been made at various times during the development of the Record of Performance testing program at this station. The earlier studies are based on fewer sire groups and are probably less reliable than the later studies.

The heritabilities obtained in the present study are based on components which have been adjusted by means of covariance for age at weaning or for age or final weight at the end of the feedlot period. They agree in most instances with those found by Knapp and Clark (1950).

Birth weight was more highly heritable (0.72) than in previous studies. Other estimates of heritability were 0.23, 0.42, and 0.34 by Knapp and Nordskog (1946a), 0.29 and 0.11 by Dawson *et al.* (1947), 0.45 by Knapp and Clark (1950), 0.45 and 1.00 by Gregory *et al.* (1950), and 0.22 by Burris and Blunn (1952). Botkin and Whatley (1953) found a repeatability of 0.18.

Heritability of weaning weight (0.23) was in the general range of previous estimates. They were 0.12 and 0.30 by Knapp and Nordskog (1946a), 0.26 and 0.52 by Gregory *et al.* (1950), and 0.28 by Knapp and Clark (1950). Estimates of repeatability were 0.51 by Koger and Knox (1947), 0.52 by Koch (1951), and 0.43 by Botkin and Whatley (1953).

Estimates of the heritability of gain (0.60) and final weight (0.84) were only slightly lower than in previous studies. For gain they were 0.99, 0.46, and 0.97 by Knapp and Nordskog (1946a), 0.65 and 0.77 by Knapp and Clark (1950), and 0.70 by Knapp and Clark (1951), and for final weight they were 0.81, 0.69, and 0.94 by Knapp and Nordskog (1946a), 0.72 by Knapp and Clark (1947), and 0.86 and 0.92 by Knapp and Clark (1950). The analysis of covariance was used to remove the effects of differences in age on final weight at completion of the test. This represents an over-correction due to the fact that the regression is based on the overall gain from birth to the end of the feedlot period. These estimates indicate that the rate of progress in a selection program will be rapid when selection is based on the individual's own record.

Efficiency (0.22) was less heritable than in the previous study of Knapp and Nordskog (1946a) which gave 0.75, 0.54, and 0.48 as estimates. In the present study, steers were individually fed through 1946, and were group fed subsequently. Efficiencies of feed utilization were computed for individual animals in the former period, but not in the latter. Consequently, the degrees of freedom available for estimating differences between steers within sire groups are limited.

Th heritability of slaughter grade (0.42) agrees with the estimate of 0.45 by Knapp and Clark (1950), but is slightly less than the 0.63 by Knapp and Nordskog (1946b). The estimate for carcass grade (0.16) is much less than the 0.84 obtained by Knapp and Nordskog (1946b), and the 0.33 by Knapp and Clark (1950). Selection for degree of finish would be more effective than selection for carcass grade if the latter could be expressed in the individual. Since it is impossible to obtain this characteristic in the individual, selection must be based on close relatives. Improvement would be only slight if conscious selection were practiced for this characteristic.

The heritability of shrink was 0.91. The actual data were expressed in pounds of shrink. They were adjusted to a constant final weight by the analysis of covariance. This high heritability indicates that shrink was relatively uniform among the steer progeny of the various sire groups, but that important differences between sire groups existed. The heritability of dressing percentage (0.73) is in striking contrast to the 0.01 reported by Knapp and Nordskog (1946b).

Color of eye muscle (0.31) and thickness of fat (0.38) were only slightly heritable. No previous estimates of heritability were available

for these characteristics. The value for area of eye muscle (0.72) agrees with the 0.69 by Knapp and Nordskog (1946b) and the 0.68 by Knapp and Clark (1950).

The present estimates of the heritability of the various growth characteristics indicate that rapid improvement can be made in the individual characteristics. Efficiency of feed utilization was less highly heritable than the other growth characteristics. This trait is unique in that it is the only character in the present study in which line differences were statistically significant. This fact suggests that lines of relatively high efficiency may be developed.

Heritabilities of the various carcass characteristics varied greatly. Improvement in most of these traits is possible, but will be slow since these characteristics cannot be observed directly in the animal selected for breeding stock. Since heritability is low, selection based on traits other than area of eye muscle or dressing percentage does not seem feasible.

Since the heritabilities of most growth characteristics are relatively large, the individual's own phenotype should be the most important basis for selection. Collateral relatives and progeny test should be used only to make selection more accurate. The sib or progeny test must be used in the selection for carcass characteristics. The sib test is advantageous in that it does not increase the generation interval, but is generally less accurate than the progeny test when based on few individuals.

Summary

The present study is a summary of the data collected during ten years (1942–1951) of Record of Performance testing at the U. S. Range Livestock Experiment Station at Miles City, Montana. The data consist of records of 635 steers from grade cows mated to 88 sires from nine lines. The characteristics studied were: birth weight, weaning weight, final weight at the completion of the feedlot period, gain in the feedlot, efficiency of feed utilization, shrinkage in shipment, slaughter grade, dressing percentage, carcass grade, color of eye muscle, area of eye muscle, and thickness of fat.

Intra-year and -line heritabilities were computed by the method of paternal half-sib correlation. The effects of differences between years, between lines within years, and between sires within lines were separated by the analysis of variance, and their relative magnitude was estimated,

while the effects of age at weaning, and age and final weight at the completion of the feedlot period were removed by covariance.

The following results were obtained:

1. The effects of differences

(a) between years were highly significant for all characteristics.

(b) between lines within years were significant for efficiency of feed utilization.

(c) between sires within lines were not significant for carcass grade, were significant for weaning weight, and were highly significant for the other characteristics.

2. The following estimates of heritability were obtained: birth weight 72, weaning weight 23, gain in the feedlot 60, final weight at end of feedlot period 84, efficiency of feed utilization 22, slaughter grade 42, shrink 91, dressing percentage 73, carcass grade 16, color of eye muscle 31, area of eye muscle 72, and thickness of fat 38.

3. Selection for most growth characteristics should be based on the individual's own record, while selection for carcass characteristics must be based on sib or progeny test.

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INFLUENCE OF SEX, SEASON OF BIRTH AND AGE OF DAM ON ECONOMIC TRAITS IN RANGE BEEF CATTLE [1] [2]

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THIS study seeks to evaluate the influence of sex, season of birth, and age of dam on birth weight, weaning weight, gain from birth to weaning, weaning score, fall yearling weight, and fall yearling score of range beef cattle. Evaluating the average influence of identifiable sources of variation and adjusting the observations for them amounts to controlling, statistically, a portion of the variation. Statistical control may not remove all variation from a given source because of errors made in evaluation. If the effect varies from one observation to the next, only the average effect will be removed by statistical control. Even so, any variation removed increases the accuracy with which the real differences between animals can be assessed.

It has long been noted that bull calves are significantly heavier at birth than heifers. In the dairy breeds this difference has been variously reported as 1 to 8 lb. with an average of about 4.8 lb. in favor of bull calves (Braude and Walker, 1949; Eckles, 1919; Fitch, McGilliard and Drum, 1924; Knapp, Lambert and Black, 1940; McCandlish, 1922; and Tyler, Chapman and Dickerson, 1947). Among the beef breeds the sex difference reported ranged from 4.2 to 5.8 with bull calves averaging about 4.7 lb. heavier than heifers (Burris and Blunn, 1952; Dawson, Phillips and Black, 1947; Gregory, Blunn and Baker, 1950; Knapp, Lambert and Black, 1940; Knapp *et al.*, 1942; and Woolfolk and Knapp, 1949). The difference between male and female beef calves at weaning has been reported as 22, 23, 32, 28, and 3 to 14 lb. by Knapp *et al.* (1942); Koch (1951); Koger and Knox (1945); Woolfolk and Knapp (1949); and Gregory, Blunn and Baker (1940), respectively.

The workers studying the influence of age of dam on birth weight concluded in general that the birth weights of calves increased with

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age of dam until 6 to 8 years of age, the greatest change being between the first and second calves (Braude and Walker, 1949; Burris and Blunn, 1952; Eckles, 1919; Fitch, McGilliard and Drum, 1924; Knapp, Lambert and Black, 1940; and Knapp *et al.,* 1942). Knapp *et al.* (1942) reported the weaning weight of calves increased with age of dam up to six years of age and then declined. Knox and Koger (1945) found that weaning weight increased with age until cows were seven years old and then declined slightly.

Data

The data used in this study are birth weights, weaning weights and scores, and fall yearling weights and scores, from 5952 Hereford calves raised at the U. S. Range Livestock Experiment Station, Miles City, Montana, during the period 1926 to 1951. The scores were available only from 1939 to 1951. The fall yearling weights and scores are from heifers approximately 18 months old. U.S.D.A. scoring form A.H. No. 522 was used. The scoring system lists five grades corresponding to the top five U.S.D.A. market feeder grades. The difference between grades is 1.5 units.

Results and Discussions

Sex

In this study the influence of sex was evaluated for birth weight, weaning weight, and weaning score. The data were classified according to the year the calf was born, the age of the dam, and the sex of the calf. The average values for the males and females, along with the average difference, were computed for each age of dam. These averages and the number of calves in each group are presented in table 1. No trend in the average differences between the sexes with increasing age of dam is apparent for any of the traits (table 1, last column). The sex influence was, therefore, evaluated from the average difference over all ages of dams. The average weaning weights for males includes both bulls and steers. Separate correction factors for bulls and steers were not calculated because it was not possible to separate the physiological effects of castration from the effect that selection for size may have had in deciding which bulls to castrate.

The average differences between male and female calves for birth weight and weaning weight were 5.6 and 26.2 lb., respectively, in favor

of the male calves. These values are intermediate in the range of sex differences reported in the literature. The small difference of .13 units in favor of male calves found for weaning score is not considered of

TABLE 1. INFLUENCE OF SEX ON BIRTH WEIGHT, WEANING WEIGHT, AND WEANING SCORE FOR CALVES FROM COWS OF VARIOUS AGES

Age of Cow	Males		Females		
	No. of Calves	Av. birth Weight	No. of Calves	Av. birth Weight	Av. Difference
(years)		lb.		lb.	
3	703	75	693	70	5
4	619	78	605	73	5
5	468	80	465	73	7
6	383	80	367	74	6
7	304	79	290	75	4
8	246	79	234	75	4
9	193	80	167	75	5
10	107	80	108	73	7
Total	3023	78.4	2929	72.8	5.6±.20
		Av. Weaning Weight [1]			
3	703	363	693	339	24
4	619	388	605	361	27
5	468	399	465	373	26
6	383	408	367	380	28
7	304	406	290	387	19
8	246	414	234	380	34
9	193	406	167	377	29
10	107	407	108	380	27
Total	3023	392.6	2929	366.4	26.2±1.10
		Av. Weaning Score			
3	484	6.8	465	6.7	.1
4	413	7.1	403	7.0	.1
5	295	7.3	309	7.1	.2
6	236	7.4	224	7.4	.0
7	182	7.5	178	7.4	.1
8	161	7.6	142	7.2	.4
9	120	7.4	102	7.3	.1
10	68	7.4	63	7.3	.1
Total	1959	7.21	1886	7.08	.13±.02

[1] All weaning weights are adjusted to 182 days of age.

practical importance as it is less than one-fifth of a standard deviation although the difference was statistically significant. In the scoring system used, .13 represents less than one-tenth of a grade.

Season of Birth

Most of the calves in this study were born during the months of April and May, but at weaning time all were weaned on the same day. The influence of season of birth was studied by regressing the various traits on age at weaning. The data were separated into subclasses of calves born in the same year and out of cows of a given age. Thus, the calves born in 1930 would be divided into subclasses according to the age of their dams. Analyzing the data within subclasses removes the average effects that years and ages of dam have on the traits.

The regression of birth weight on weaning age was —.08 lb. per day (±.01). Thus, calves born later in the calving season were slightly heavier at birth. This slight difference could be due to better pasture conditions or possibly to the weight difference caused by variation in gestation lengths of cows.

The regression of gain from birth to weaning (obtained by multiplying actual rate of gain by 182) on weaning age was —.04 lb. per day (±.05). This regression may be interpreted in several ways. If calves grow linearly with age, it indicates that calves born early in the season did not grow quite as rapidly as calves born later, a view that is contrary to popular opinion. If rate of gain decreases slightly with age, a slight negative regression would be expected even though the calves were following the same growth pattern. An opposite expectation occurs if the curvilinear growth, observed by some workers in range cattle, is due to the effects of the season. From general considerations it seems that both of these influences are operative. The net result of these opposing influences may have caused the growth observed in these data to be near linear. The fact that the regression was not significantly different from zero, statistically or practically, does indicate that the difference between the growth rates of the early and late calves is not as important as many people, including the authors, have thought. Different results might be obtained on data covering a wider range of birth dates. The conflict between the facts found here and the impression that earlier calves appear to do better is no doubt due to the inability of a person to adjust mentally for differences in age when examining a group of calves of mixed ages. Because the growth curve of these cattle is confounded with seasonal differences, the linear growth found here may not be representative of the growth pattern of calves in other localities.

The regression of weaning score on weaning age was .01 unit per day (±.005), indicating that early calves tend to score a little better than

those born later in the season. The older calves would generally be heavier and more mature, thus scoring higher. In another analysis of these data the correlation between weaning weight and score was .64.

The standard deviation for age at weaning was 13 days and the standard deviations of birth weight, gain from birth to weaning, and weaning score were 8 lb., 44 lb., and 0.7 unit, respectively. Therefore, none of the regressions are considered of practical significance.

Age of Dam

Cows influence the preweaning growth of their calves both by the genes transmitted to the calf and by the maternal environment provided to weaning. Presumably, changes in size, weight, and physiological function which accompany aging might be expected to influence this environment and consequently have a direct effect on birth and weaning weight. Because the maternal environment which affects weaning weight may also influence weaning score, fall yearling weight, and fall yearling score, the influence of age of dam on these traits was considered.

Two methods of calculating correction factors for age of dam are to compare averages of all records made at each age (Method A) and to compare records made by the same cow at two different ages (Method B). Because of possible selection, each method may be biased from the true age effect and the biases are in opposite directions. A thorough discussion of these biases is given by Lush and Shrode (1950). Only a brief discussion of the nature of the biases is included here.

If some of the cows with low producing ability are culled at each age, then at each succeeding age the group would contain a larger share of high producing cows and a smaller share of low producing cows than at the younger ages. Consequently, an age of dam correction factor computed by comparing the average production from cows at older ages with the average production from all cows at younger ages (Method A) would be biased upward from the true age effect.

Method B seeks to avoid the effects of selection of cows by comparing records at two ages on the same cow, thus eliminating differences between cows. Yet, this method also introduces a bias from selection because of the incomplete repeatability of records by the same cow. Suppose at a given age we cull the cows having the lowest records. The group of cows saved will have in it a few cows that were inherently below the culling level but, because of fortuitous environmental conditions, had acceptable production records. Conversely, the group culled will contain a number of cows whose inherent abilities were above the

culling level but, because of unfavorable environmental circumstances, their actual production fell below the culling level. Therefore, the inherent level of production in the selected group is lower than the observed level of production. In subsequent years the cows are subjected to new sets of environmental conditions and the observed production level will regress toward the inherent level of the group. The average comparison of two records by the same cow will be biased downward from the true age effect.

A mathematical expression for the biases as given by Lush and Shrode follows:

Let N = number of cows having records at a given age; k = number having records at the next age; $c=N-k$ = number of cows making records at the first age but failing to make records at the next age; K = the average production of the k cows at the first age; K' = the average production of the k cows at the next age; C = the average production of the c cows at the first age; C' = the average production the c cows would have had at the next age if they had been saved.

The true change due to age would be

$$\frac{kK'+cC'}{N}-\frac{kK+cC}{N}=\frac{k}{N}(K'-K)+\frac{c}{N}(C'-C).$$

The apparent change due to age as calculated by Method A is $K'-\frac{kK+cC}{N}$. The bias is the difference between the true change and the apparent change which equals $\frac{c}{N}(C'-K')$. The apparent change with Method B is $K'-K$ and the bias is $\frac{c}{N}[(C'-K')-(C-K)]$. The difference between the methods is $\frac{c}{N}(C-K)$.

If p is the regression of observed producing ability on real producing ability $C'=p(C-\text{herd average})+\text{herd average}$ and $K'=p(K-\text{herd average})+\text{herd average}$. $C'-K'=p(C-K)$. The bias in Method A equals $\frac{c}{N}p(C-K)$ and in Method B$-(1-p)\frac{c}{N}(C-K)$. The real age effect lies between the values found by Methods A and B. The ratio of the biases is $\frac{p}{1-p}$.

The influence of age of dam on birth weight, weaning weight, weaning score, fall yearling weight, and fall yearling scores was evaluated both by Method A and Method B. The influence of age of dam according to Method A was determined by averaging the production records

for each age of cow and comparing within years. These values were then averaged over all years. For most traits the maximum production appeared to be at six years of age and this age was selected as a base for comparisons. Method B involved the comparison of two records from the same cow at different ages. In this study all cows having records at two consecutive ages were selected and the differences com-

TABLE 2. NUMBERS OF RECORDS USED IN ESTIMATING THE INFLUENCE OF AGE OF COW

Method A

Cows Having Calves at Each Age

Item	Age of Cow in Years							
	3	4	5	6	7	8	9	10
Birth wt.	1395	1221	927	752	592	478	359	223
Weaning wt.	1395	1221	927	752	592	478	359	223
Weaning score	948	817	607	467	361	305	227	143
Yearling wt.	497	450	350	324	246	198	143	95
Yearling score	313	303	256	223	161	126	100	65

Method B

Cows Having Calves at Each Pair of Ages

	Ages of Cow in Years						
	3–4	4–5	5–6	6–7	7–8	8–9	9–10
Birth wt.	925	763	603	484	378	301	183
Weaning wt.	925	763	603	484	378	301	183
Weaning score	539	437	334	230	183	155	93
Yearling wt.	137	106	62	95	66	50	33
Yearling score	85	71	40	60	37	31	20

puted. This grouping gave the maximum number of comparisons since some cows were culled at each age. Year effects were removed using the method of least squares, adjusting for differences between years and differences between ages simultaneously. The number of cows having calves at each age in the case of Method A and the cows having calves at each pair of ages in the case of Method B are shown in table 2.

The true age effect was shown above to be between the two methods as $\frac{p}{1-p}$. If one could evaluate p, an unbiased estimate could be obtained by combining the estimates derived by Methods A and B. In these data cows were culled on the basis of weaning weight and score of their calf, conformation of the cow, defects, injuries, failure to calve for two consecutive years, and old age. Of the production characters

considered in this study only weaning weight and score were used as a basis for selection of cows once they were in the breeding herd, with weaning weight being given the most consideration. Assuming that selection for production was based on weaning weight and that weaning weight was not correlated with the other traits considered in culling, such as conformation of the cow, defects, and injuries, p is approximated by the repeatability of adjacent weaning weights. The repeatability of adjacent records was used because the decision to keep or to cull was made each year. The repeatability of adjacent weaning weights slightly underestimates the regression of one year's record on the next because weaning score did receive some attention, and it is positively correlated with weaning weight. From another study of these data p was estimated as .46. The difference between Methods A and B may then be proportioned as $\frac{p}{1-p}$. The age correction factors derived from Method A, Method B, and the combined estimates are presented in table 3. Figure 1 presents the same information graphically. Six years was used as the standard age because that appeared to be the age of maximum production.

The estimates obtained by combining the information from Method A and Method B represent the best estimates found in this study of the additive age effect. The following discussion pertains to these estimates as given in table 3 and figure 1. The pattern of the effects of age of dam as illustrated in figure 1 indicates that maximum production was reached at six years of age for birth weight, weaning weight, and weaning score. Gifford (1953) reported that milk production in Hereford cows was greatest at six years of age.

The largest difference for birth weight was between the ages of 3 and 4 years. In applying the corrections it would seem that adding correction factors for the ages of 3, 4, and 10 years or older would remove most of the variation due to age of dam in birth weight.

Weaning weight also showed the largest change between the ages of 3 and 4 years. The sizable differences found indicate that correction should be made for all ages of dam. In these data, the value for 7-year-old cows deviates from the pattern formed by the other ages. As there was no apparent biological reason for this, one-half of the difference between the correction for 6- and 8-year-old cows (3.0 lb.) is suggested as the correction to be added to the weaning weights of calves from 7-year-old cows. The linear interpolation is well within the sampling errors computed.

The correction factors for weaning score follow closely the pattern set by weaning weight. As with weaning weight the correction factor for 7-year-old cows should probably be changed to half the difference be-

TABLE 3. CORRECTION FACTORS FOR AGE OF COW

Age of Cow	Birth Weight	Weaning Weight	Weaning Score	Fall Yrlg. Weight	Fall Yrlg. Score
	Method A (Comparing Ages with Years)				
Years	lb.	lb.	units	lb.	units
3	4.7±.4	46±2	.6±.04	29±4	.1±.05
4	2.1±.4	21±2	.3±.04	19±4	.1±.05
5	0.2±.4	8±2	.2±.04	5±4	.0±.06
6	0.0	0	.0	0	.0
7	0.4±.5	8±2	.1±.05	2±5	.0±.06
8	0.4±.5	2±3	.0±.05	−3±5	.1±.07
9	−0.1±.5	7±3	.1±.06	−5±6	.0±.08
10	1.4±.6	15±3	.3±.07	−11±7	−.1±.09
	Method B (Comparing Consecutive Calves Out of the Same Cow				
3	3.6±.4	35±2	.5±.04	18±8	.1±.1
4	1.1±.4	13±2	.2±.04	7±8	−.1±.1
5	0.3±.4	4±2	.1±.05	1±9	−.1±.2
6	0.0	0	.0	0	.0
7	0.0±.4	5±2	.1±.06	−1±8	.1±.1
8	1.6±.5	11±2	.2±.06	−7±8	.1±.1
9	.9±.5	19±2	.2±.06	9±9	.0±.2
10	3.4±.6	35±3	.5±.07	18±11	.0±.2
	Combined Estimates, where $\frac{1-p}{p}=.54/.46$				
3	4	41	.6	24	.1
4	2	18	.3	13	0
5	0	6	.2	3	0
6	0	0	.0	0	0
7	0	3[1]	.0[1]	2[1]	.1
8	0[1]	6	.1	4[1]	.1
9	0	12	.2	7[1]	0
10	2	24	.4	14[1]	0

[1] Interpolated values as outlined in discussion.

tween 6- and 8-year-old cows (.05 unit). Because of differences between systems of scoring used by various workers it may be advantageous to convert the values found here into a more general expression. As previously stated, the scoring system used lists five grades and the difference between grades is 1.5 units. The correction factors reported in table 3 can be converted to fractions of a market grade by dividing the correction by 1.5.

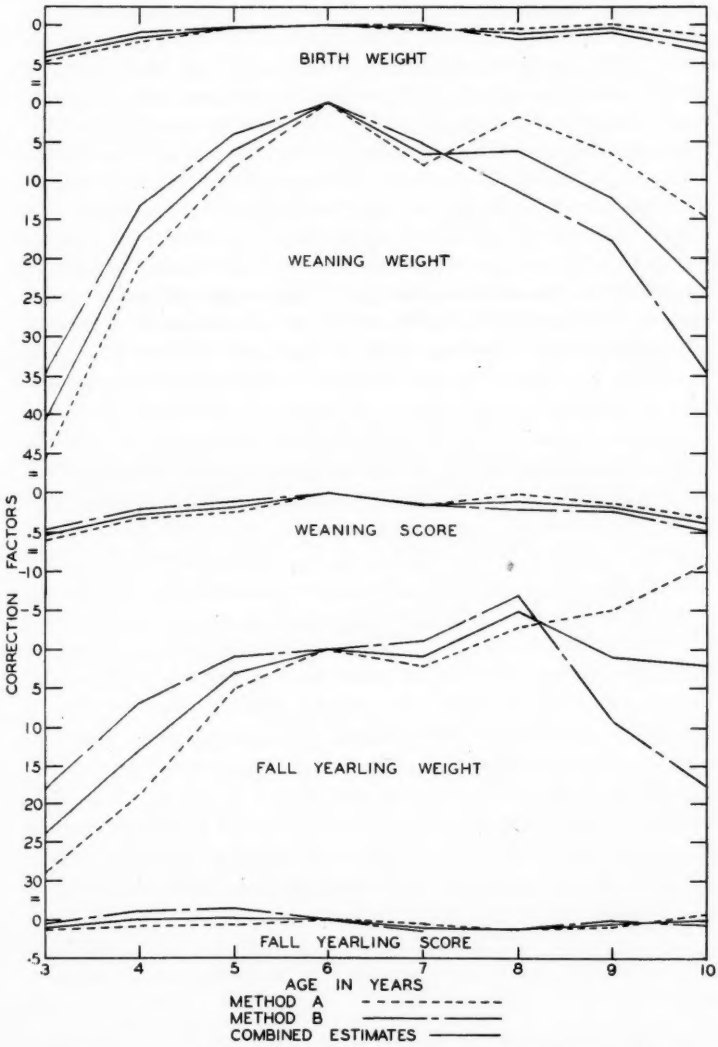

Figure 1. Changes in characteristics of the offspring with change in age of the cow.

The fall yearling weights of cattle out of 3-year-old cows may be expected to be less than those of yearlings out of 4-year-old cows, for example, because they weighed less at weaning. If the effect of age of dam on weaning weight was independent of subsequent gains, we would expect the same difference at fall yearling age as at weaning. Actually there was less difference at yearling age than at weaning. The smaller difference at yearling age probably illustrates the tendency of calves to grow more rapidly following periods of limited feed supply, in this case possibly because of differences in milk supply. It would seem that the pattern for fall yearling weight should follow that of weaning weight, but the differences between the ages would be smaller. The patterns for weaning weight and fall yearling weight were similar up to 6 years of age. After that age, weaning weight declined, but fall yearling weight increased. The reason for this discrepancy could not be ascertained. Possibly selection was not discounted properly in combining the values of Methods A and B. The discrepancy could also be due to sampling error as the standard errors for the fall yearling weight correction factors are quite large. In applying these correction factors, two alternative courses of action are suggested. One method is to use the correction factors shown in table 3 for the ages up to 6 years and make no correction for ages over 6 years. The alternative suggestion is to use the factors in table 3 for ages up to 6 years and then take the correction factors for weaning weight as a guide in deriving the corrections of fall yearling weight for the ages 6 through 10 years. The fall yearling weight correction factors observed at the ages between 3 and 6 years averaged 60 percent of the corresponding factors for weaning weight. Using 60 percent of the weaning weight correction as a guide, the correction factors for fall yearling weight at the ages of 7, 8, 9, and 10 years would be 2, 4, 7, and 14 lb. respectively. This procedure is certainly subject to question, yet it seems better than no correction at all. Similar studies on other data are necessary to indicate the true nature of the age of dam effect on fall yearling weight.

The correction for fall yearling score does not follow the pattern of weaning weight, but it seems too small to be of any practical importance.

Summary

The purpose of this study was to evaluate the influence of sex, season of birth, and age of dam on birth weight, weaning weight, gain from

birth to weaning, weaning score, fall yearling weight, and fall yearling score for range Hereford cattle.

Male calves were 5.6 lb. heavier at birth and 26.2 lb. heavier at weaning than heifer calves. The sex difference in weaning score was negligible.

The regressions of birth weight, gain from birth to weaning, and weaning score on weaning age (the inverse of time born during the calving season) were —.08 lb. per day, —.04 lb. per day, and 0.1 unit per day, respectively. All regressions were considered to be too small for practical importance.

The age of dam had a marked influence on all traits studied except fall yearling score. The cow's production with regard to birth weight, weaning weight, and weaning score increased steadily from 3 to 6 years of age and then declined. Additive correction factors are presented for adjusting records to mature (6-year-old) basis.

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A BIOPSY TECHNIQUE AND ITS USE IN STUDYING CHANGES IN MUSCLE DURING FATTENING[1]

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MANY investigations in the field of farm animal research are limited in their interpretation because of the small number of animals in any particular treatment. In sheep nutrition and carcass research, and more commonly in that of swine, siblings may be used to reduce the experimental error due to individual variation, but this experimental design can seldom be used where the research involves beef animals.

In an attempt to reduce the variation due to inherent differences in individual animals, the biopsy technique described below was developed to study the effect of fattening on the connective tissue content of steers. Merkel *et al.* (1954) have devised a somewhat similar technique for removing a muscle sample for the longissimus dorsi of hogs.

The purpose for developing this technique was to afford an opportunity to make a more detailed study on the effects of fattening on the tenderness of beef.

Technique

The animals were restrained in a dehorning chute and anesthetized extra-durally between the first and second coccygeal vertebrae with procaine (10–15 cc. of a 2.5 percent solution). The operative area over the semimembranosus-semitendinosus septum was washed and shaved. The exact location of the septum was determined by palpation and an eight inch incision made over it. By blunt dissection a section of the semimembranosus muscles two inches square was separated. This tissue was then cut perpendicular to the muscle fibers yielding a rectangular portion of muscle approximately two inches square and four inches long (150–400 gm.). A sample being removed from a steer is shown in figure

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1. The exposed tissue was dressed with urea and a sulfa drug, the skin sutured and iodine and collodion bandages were applied. The sutures were removed after 2 weeks and the incision was fully healed in 3 to 4 weeks.

Figure 1. Rectangular portion of semimembranosus muscle being removed from round.

Experimental

In an attempt to evaluate the biopsy technique as a method for the study of the effect of fattening on tenderness, eight Commercial grade steers weighing between 700 and 800 lb. were used in each of two replicate experiments. Three or 4 days after the steers were placed on a fattening ration control samples from the left semimembranosus muscle were taken according to the method described above.

The samples were stored at 34° to 36° F. for 24 hours and then tested for tenderness with a Warner-Bratzler shear machine. In these experiments the biopsy sample taken from the steers necessitated using a core smaller than the recommended one inch size. In an effort to get uniform small cores, the apparatus shown in figure 2 was designed. The sample was placed with the fibers running parallel to the one-half inch

semicircle in the lower block. Weights totaling 20 lb. were then placed over the corner pins and the core removed with a sharp knife. Because the biopsy sample was limited, shear tests were made on the raw sample, recognizing that the Warner-Bratzler shear machine was designed for and gives a truer test of tenderness on cooked samples.

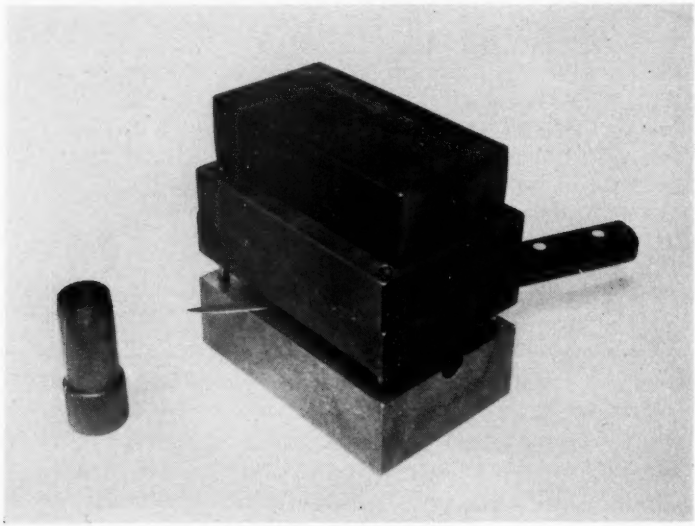

Figure 2. Apparatus assembled for cutting modified core of meat. Borer for one inch core shown at left.

The steers in experiment I were fed a fattening ration 109 days while those in experiment II were fed 120 days before slaughter. At the time of slaughter, samples similar in size and location to the biopsy samples were removed from the right semimembranosus muscle for comparative studies.

The samples were tested for tenderness by the Warner-Bratzler shear machine by use of the technique described above. Additional data as regards moisture, intramuscular fat, and percentage collagen were gathered, yet additional data seem necessary before any critical evaluation can be made.

Results and Discussion

The feed and gain data showed the steers in the second experiment to have gained 0.5 lb. per day faster and did so on less feed than those

in the first experiment. This difference in gain was also reflected in the carcass grade, which was about one-third of a grade higher for the faster gaining steers. These differences were thought to be due to inherent differences in the ability to utilize feed or to differences in age of the cattle.

A summary of the muscle characteristics studied in each experiment is shown in table 1.

TABLE 1. AVERAGE MOISTURE, FAT AND SHEAR VALUES OF THE SEMIMEMBRANOSUS MUSCLES OF STEERS

Characteristics Studied	Experiment I		Experiment II	
	Initial Biopsy Data	Terminal Data	Initial Biopsy Data	Terminal Data
Moisture percent	74.11	74.28	77.28	75.65*
Fat percent	2.27	2.69	0.71	2.57*
Shear value	22.2	18.7*	20.6	15.4*

* Significant at 1% level.

An inverse relationship of moisture content to fat content was found to be highly significant in the samples taken from the steers in the second experiment. Since the total intramuscular fat changed only slightly in the first experiment, it seems logical that little difference was found in the moisture content of the biopsy or terminal samples.

The shear value of meat has long been recognized as a guide toward the evaluation of tenderness. It is especially helpful in making comparative studies.

The biopsy and terminal sample shear values were quite similar in each experiment. However of equal note was the same general trend and magnitude of improvement in shear value from biopsy to terminal sample in each experiment. While differences in shear values prior to and after the feeding period were statistically significant in each case, the shear values were decreased more in the animals in experiment II. These animals likewise deposited more intramuscular fat during the feeding period. This suggests that there may be a relationship between intramuscular fat and tenderness. These findings would support the work of Husaini *et al.* (1950), Wanderstock and Miller (1948), Black *et al.* (1931), Nelson *et al.* (1930) and Trowbridge and Moffet (1932).

In view of this preliminary study it appears as if this technique will provide satisfactory samples for chemical and physical data as regards

muscle which may be helpful in understanding muscle changes that develop in our meat animals as they grow and fatten.

Summary

A biopsy technique is described by which samples of the semimembranosus muscle can be taken from cattle. Samples can be taken from the opposite muscle after experimental treatment. If experimental treatments bring about changes in this muscle in a similar manner to that of others, then it appears that the use of this technique would allow animals to serve as their own controls.

In two experiments involving 16 steers, samples taken by this technique seemed to provide satisfactory data as regards moisture, intramuscular fat and shear test values.

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ISOLATED GUT AND RUMEN MOTILITY AS AFFECTED BY EXTRACTS FROM BLOAT PRODUCING FORAGES [1, 2]

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University of Illinois [4]

THE expanded use of legumes in forage mixtures has caused bloat to become an important problem in the management of pastures used for the production of livestock. Windler (1952) made a survey and determined the value of cattle losses in Pike County, Illinois, to be over $20,000. However, this is not a satisfactory means of evaluating the total seriousness of the problem, for it does not represent the expenses involved in buying bloat preventatives or treatments, the labor lost by having to observe grazing animals, nor the losses represented by the restricted use of legumes because of the danger of bloat.

There are several theories concernig the cause of bloat most of which have been discussed in comprehensive reviews by Cole *et al.* (1945) and by Dougherty (1953). Cited in these reviews is the biochemical or toxic factor theory. It is suggested that these substances may cause partial paralysis of the rumen and thereby prevent belching. Evans and Evans (1948) suspended 2-inch lengths of rabbit intestine in aerated Tyrode solution and added various plant juices to demonstrate that intestinal motility was impaired when clover or alfalfa juice was present. Stewart (1949), using a similar technique, tested orchard grass, timothy, and Ladino clover. Clover juice alone, of these three, caused the peristalic movement of the intestine to cease. Ferguson (1948) demonstrated that lucerne juice contained a substance which inhibited the activity of the rabbit ileum *in vitro*. Further work by Ferguson *et al.* (1949) indicated this substance to be 3′4′5′-trihydroxy 7-methoxy flavone, but more recent investigation (Ferguson *et al.*, 1950) has shown it to be tricin, 5′7′4′ trihydroxy 3′5′ dimethoxy flavone. Most flavones inhibit smooth muscle activity, and for this reason these compounds are significant, and their presence may partially support the toxic substance

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theory for bloat. The past work, therefore, indicates there may be a parallelism between the muscle inhibiting activity of certain plant extracts and the expected potential of the plants to produce bloat.

The study herein reported was conducted with the idea that bloat may result because of a toxic or biochemical substance in the forage that interferes with the normal ruminal-eructation mechanism thereby resulting in an accumulation of gases in the rumen. A simple *in vitro* muscle technique was employed to assay forages for their possible bloat-producing ability. Further it was thought that if the inhibiting substance was related to bloat, ingesta from bloated animals should contain this substance while the ingesta from normal rumen contents would lack this power of inhibition. Ingesta from normal and bloated animals were, therefore, assayed for the muscle inhibiting capacity.

Experimental Procedure

Varying amounts of forage extracts and rumen contents were tested in this study by determining their effect on the motility of segments of isolated rabbit intestine. The following is a description of the technique used.

A rabbit was killed by cutting the throat, the abdominal cavity exposed, and the small intestine freed from the mesentery. The intestine was sectioned about eighteen inches posterior to the pylorus, using only the jejunum section of intestine in order to obtain a consistent reaction from the sectioned intestine. A 12 to 15-inch section of jejunum was removed and immediately washed with warm 35° C. 1:1 Tyrode-Locke solution after which the segment was cut into one-inch sections. These sections were then handled in one of the following ways: (1) a section was taken immediately from the wash solution and put in a Tyrode bath (125 ml. at 35° to 38° C.) for a motility study; (2) the sections were successfully stored for 3 to 5 hours in a well aerated 1:1 Tyrode-Locke solution held at 32° to 35° C.; (3) the sections were kept for 12- to 36-hour periods of time by putting them in a well aerated 1:1 Tyrode-Locke bath which was gradually cooled from 35° to 5° to 8° C., aeration being discontinued at approximately 15° C. When this bath was warmed, heat was applied slowly, and aeration was initiated at approximately 15° C. and continued after a temperature of 33° to 35° C. was reached. A preliminary test by aerating with pure oxygen and compressed air showed no difference between the two methods, therefore, compressed air was used in these studies.

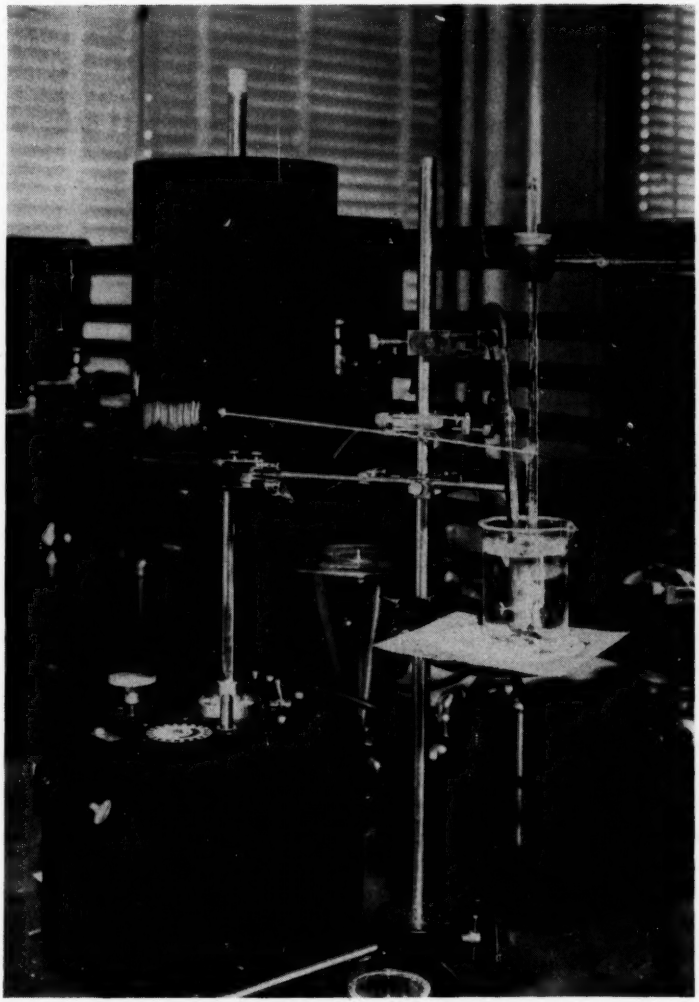


Figure 1. Intestinal motility recording apparatus.

The recording of the intestinal activity was made possible by attaching one end of the section of intestine to a stationary clamp and the other end to a clamp connected to a recording lever of a suitable record-

ing device. In this study, a kymograph with a 5-second interval timer was used for the tracings. The intestine was completely immersed in Tyrode bath at all times, and the bath was aerated at a rate that would not interfere with the intestinal activity or the movement of the recording lever. Usually the section of intestine became active in a few minutes, and in some instances one section of intestine was used for

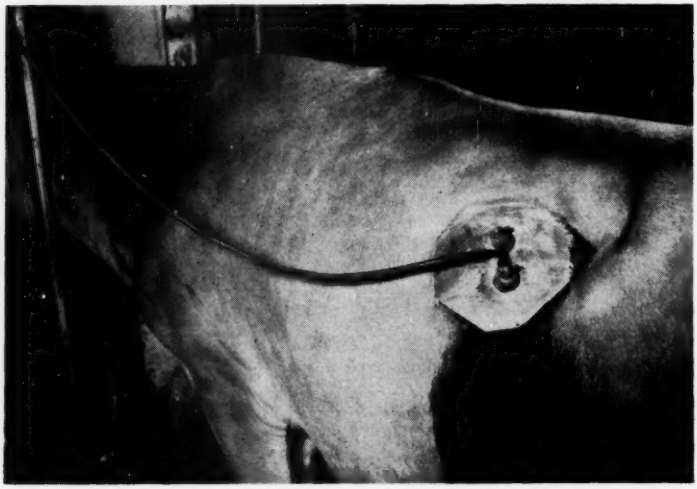


Figure 2. Fistulated cow used for intra-ruminal tracings.

several tracings. After each contamination with a test material the Tyrode bath was removed, the intestinal section was washed with fresh Tyrode solution, and then placed in a fresh Tyrode bath before tracings were continued. A view of the apparatus used in these tests is shown in figure 1.

For the studies involving the intact animal a lactating Jersey cow with a ruminal fistula was used, figure 2. Intra-ruminal pressure changes were recorded by attaching one end of a ¼-inch rubber pressure tubing directly to a valveless inner-tube stem securely fastened in the center of a fistula plug, while the other end of the tubing was attached directly to the recording device, thereby forming a closed system in which the rumen pressure changes were detected, figure 3.

The forage samples tested were collected both from pastures known to be causing acute bloat in cattle and from pastures not causing bloat.

The samples were hand-picked in an effort to simulate a grazing animal and to also collect a representative sample of pasture. Samples of forage from pastures causing bloat were collected either at the time bloated cattle appeared in the herd or immediately after actual death losses occurred. These samples were taken immediately to the laboratory and finely ground in a large mill and pressed with a hydraulic press to extract the juice. The extracted juice collected usually amounted to about

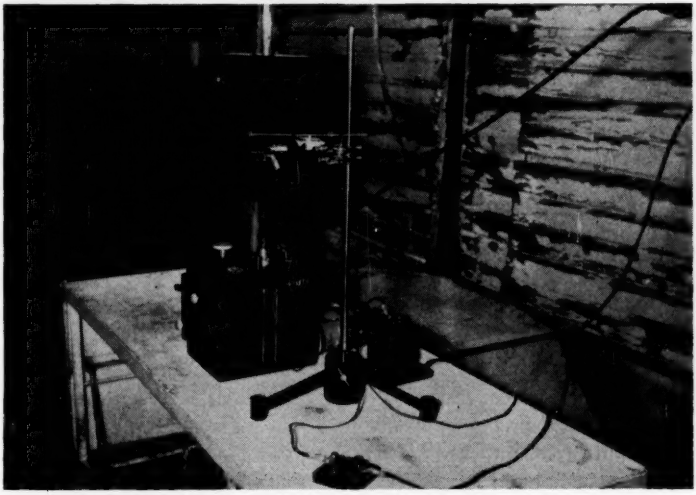
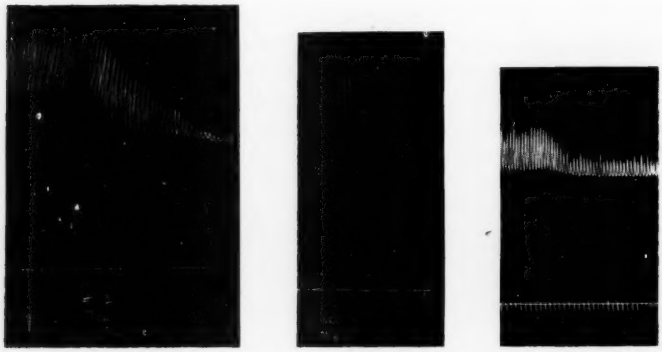


Figure 3. Recording apparatus used for intra-ruminal tracings.

one-third of the original weight of the forage sample. Samples of the juice were used either for immediate motility tests, given orally to experimental animals, or frozen immediately at $-10°$ C. and stored for future studies. The rumen contents studied were collected from normal cattle on various rations at slaughter as well as from cattle immediately after death due to acute bloat while grazing. The rumen contents tested consisted of aliquots of the liquid portion.

Results and Discussion

Considerable variation was noted in the effect of extracts of various forages on the motility of the isolated rabbit intestine. In general, however, the extracts from forages known to have caused acute bloat in cattle caused a marked inhibition in the activity of the rabbit intestine.

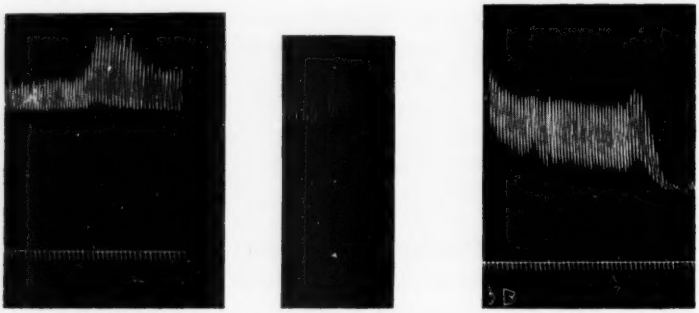


Ladino Clover A | Ladino Clover B | Sweet Clover

Figure 4 shows a typical kymographic tracing of some of the forages tested which caused bloat. The test substance and amount used are given with each tracing. Extracts from grasses usually caused only minor changes in the activity of the isolated gut.

Figure 5 shows kymographic tracings of aliquots of the liquid portion of rumen contents of cattle that died of acute bloat while grazing. The marked inhibition was constant in samples collected from three animals. The rumen liquid from cattle fed dry lot rations or cattle grazing grass pastures did not produce any abnormal effect on the motility of the isolated gut.

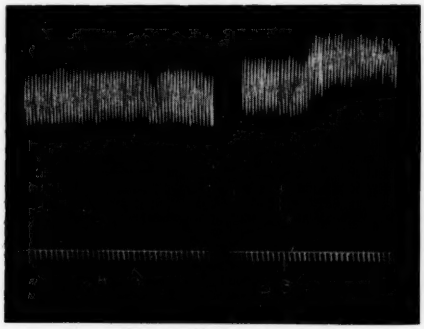
The kymographic tracing of the intra-ruminal pressure of a fistulated Jersey cow fed a grain ration and allowed to graze a predominantly



Red Clover | Alfalfa | Alsike

Figure 4. The effect of legume juices on the activity of isolated smooth muscle.

grass pasture is given in figure 6. The small notch immediately after the large rumen contraction indicates eructation. This cow was observed to eructate about four times every five minutes. When 8 lb. of

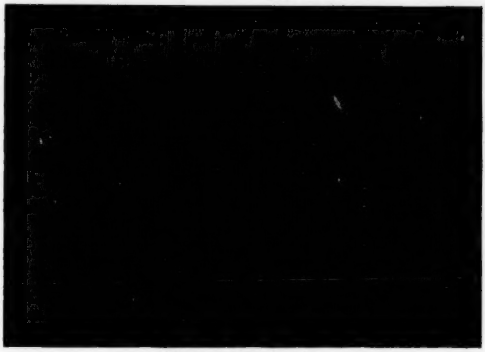


A Rumen Contents B

Steer on full feed of grain.

Heifer on grass pasture.

Rumen Contents
Cow died from bloat.
Alfalfa, Timothy
Ladino Clover



Rumen Contents
Steer died from bloat.
Alfalfa-Ladino clover pasture

Figure 5. The effect of rumen contents on the activity of isolated smooth muscle.

Ladino clover juice were introduced directly into the reticulum and anterior blind sac of the rumen there was an inhibition in eructation as indicated in figure 6. On close observation eructation occurred only three times during a period of more than thirty minutes after the extract was given. Rumen contractions were not noticeably affected. This extract

was prepared from a sample of Ladino clover that was kindly supplied by Dr. B. F. Barrantine of the Mississippi State College Experiment Station. This clover was known to produce bloat in cattle under his experimental conditions. In our laboratory this extract caused marked inhibition of rabbit intestine motility. Four pounds of this extract given orally to a mature ewe produced acute bloat and death in eleven minutes.

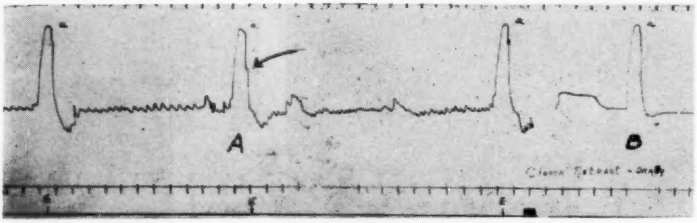


Figure 6. The effect of Ladino clover on the intra-ruminal pressure curve before and after adding Ladino clover juice.

In attempts to characterize the active substance, or substances, the property that inhibited the motility of segments of rabbit intestine was not altered on freezing and storing at $-10°$ C. for four weeks, or on heating at 100° C. for five minutes. It was also noted on one sample that the active substance was dialyzable through a semi-permeable cellophane membrane.

It was apparent in these studies that extracts from bloat-producing forages and samples of rumen contents from cows that died due to acute bloat have the property of inhibiting the action of isolated segments of rabbit intestine. Limited data also suggested that the active substance, or substances, was stable to heat and freezing and might cause bloat in the ruminant by inhibiting the normal eructation mechanism in some manner.

These studies on the nature of acute bloat in ruminants tend to support the postulation of previous workers that a toxic or biochemical factor, or factors, in succulent, lush growing leguminous forages is involved in inhibiting the eructation mechanism and thereby causing the accumulation of gas in the rumen. The observation in the field on (acute fatal) bloat in cattle and on the experimentally produced bloat in the ewe, of which severe distention of the rumen was not always present, would tend to suggest that death of the animal may in some instances be due to disturbances other than the physical effects of acute distention of the rumen.

Summary

An experimental technique with preliminary information is reported that may aid in research on the pathogenesis of bloat. Samples of rumen contents from cattle dying from acute bloat and extracts from forages causing bloat were found to inhibit the motility of segments of the isolated rabbit intestine. This property was not altered by freezing, heating, or storing the sample. An extract of Ladino clover that was known to cause bloat inhibited eructation in a fistulated cow.

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THE INFLUENCE OF HIGH SODIUM CHLORIDE INTAKES BY FATTENING SHEEP AND CATTLE

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THIS paper is one of a series of experiments conducted by the California Experiment Station on high sodium chloride intakes of animals (Weir and Miller, 1953; Weir and Torell, 1953; Meyer and Weir, 1954; Meyer, 1954; Meyer *et al.*, 1955). Not only is salt used to regulate the consumption of supplemental concentrate feed by range livestock, but its use is becoming widespread in California as a regulator of cencentrate consumption of growing and fattening ruminants on irrigated pasture. In the latter case, 8 to 10 percent salt mixed with the concentrate allows an intake of approximately 1 lb. of concentrate per 100 lb. of body weight for fattening steers on pasture. However, this level of intake is not constant, since it varies according to the quality of the pasture and needs to be shifted either up or down in order to obtain the desired concentrate intake. The following experiments were conducted because of the lack of information on the influence of high sodium chloride intakes on gains and carcass quality of fattening ruminants. This report includes a study of fattening sheep and cattle and gives results from a digestion trial.

Experimental

Two experiments were conducted with sheep, and the basal ration used for these experiments is shown in table 1. Four levels of sodium chloride were fed as follows: Lot 1, basal; Lot 2, 4.8 percent sodium chloride; Lot 3, 9.4 percent sodium chloride; Lot 4, 12.8 percent sodium chloride. All rations were ground and pelleted.

The first sheep experiment was a digestion and nitrogen-balance study composed of two separate trials. A 4×4 Latin square was the design for the first trial; a different design was used for the second trial because the first indicated that previous dietary history, as far as salt intake was concerned, might have some influence on the digestibility of the ration. The second trial was divided into seven periods, and four black-faced wethers were used for each period. The design for the second trial

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was as follows: Period 1, basal ration; Period 2, 4.8 percent sodium chloride; Period 3, basal ration; Period 4, 9.4 percent sodium chloride; Period 5, basal ration; Period 6, 12.8 percent sodium chloride; Period 7, basal ration. A 7-day preliminary and a 7-day collection period was used for these trials. The method of Lofgreen (1953) was used to determine the TDN (total digestible nutrients) of the ration.

TABLE 1. BASAL RATIONS

	Sheep	Cattle
	%	%
Alfalfa hay	84.5	35
Oat hay	——	15
Barley	15.0	35
Beet pulp molasses dried	——	15
Salt	0.5	Free choice

The second sheep experiment was a fattening trial in which 24 head of crossbred wethers and ewes were divided into four lots of six head each. They were fed the rations *ad libitum* with added sodium chloride levels as given above. Preliminary to the experiment the sheep were on a ration of hay. The weights at the start of the experiment were taken after the sheep had been shrunk overnight without feed or water. At the end of the experiment the rations of the animals were changed from the experimental feeds to alfalfa hay. They were fed alfalfa hay for two days and then shrunk overnight without feed or water in order to obtain a final weight on a shrunk basis. During the experiment the sheep were bled four times for the determination of hematocrits (packed red blood cell volume) and blood serum albumin. Albumin was separated from the blood serum by the method of Kingsley (1940) and determined as outlined by Greenberg (1929). The sheep were slaughtered at the end of the experiment to obtain dressing percentage and carcass grade. Weights were obtained on the kidney and adrenals, and the kidneys were subjected to microscopic and macroscopic examination. Stomach worm counts were made on each animal to determine if there was any influence of high salt intake.

The steer experiment was conducted at the Imperial Valley Field Station of the University of California. These steers were fed for 84 days during the summer when high environmental temperatures are common. The basal ration was pelleted and is shown in table 1. Lot 1 received the basal ration, and Lot 2 received the basal ration plus 10 percent sodium chloride. During the first 28 days, the steers developed

a great liking for the board fences and fence chewing was excessive. In order to save the fences, a pound of oat hay, in addition to the pelleted ration, was given to each steer daily and this effectively stopped the fence chewing. This brought the salt for the Lot 2 steers to 9.33 percent of the total ration. The control group (Lot 1) was also used as controls in another experiment which will be reported by Ittner *et al.* (1954). The initial weights of the steers were taken after an overnight shrink without feed or water. At the end of the experiment the steers were all placed on the Lot 1 ration for two days and then shrunk overnight without feed or water before the final weighing was made. The steers were slaughtered at the end of the experiment to obtain dressing percentage and carcass grade.

TABLE 2. DIGESTION AND NITROGEN-BALANCE TRIAL

Category of Interest	Lot 1 0.66% NaCl	Lot 2 4.8% NaCl	Lot 3 9.4% NaCl	Lot 4 12.8% NaCl
No. of trials	8	8	8	8
Total digestible nutrients, basal ration, %	57.6	58.4	56.2	56.1
Nitrogen digested, %	75	73	73	75
Nitrogen retained, %	10.8	12.4	11.4	14.4

Results

The digestion and nitrogen-balance trial (table 2) does not indicate a statistically significant influence of high sodium chloride intake on TDN, digestibility of the protein, or nitrogen retention. These data include some of the results reported previously in an abstract by Meyer *et al.* (1953). As reported at that time, NaCl seemed to have some effect on nitrogen balance. The digestibility results of that experiment were indefinite and neither confirmed or denied the contradictory findings of other workers. Riggs *et al.* (1953) had shown high salt intakes to have a stimulatory effect on digestibility of the ration, while Archer *et al.* (1952) and Cardon (1953) had found no influence of salt on digestibility. The work reported here confirms the latter findings that high sodium chloride intakes do not influence ration digestibility.

No detrimental effects from high salt intake were noted during the fattening trial with lambs (table 3). The daily gains were equivalent for all groups. There was some decrease in daily ration intake by the groups on the higher salt intakes; however, this did not influence the

average daily gain. Carcass quality as measured by grade and dressing percentage was as high for the sheep on the high salt intakes as for those on the basal ration.

Kidney weights did not increase when salt was added to the ration at the level of 4.8 percent. However, increasing the salt level to 9.4 percent and 12.8 percent increased the size of the kidney. Examinations of the kidney revealed no pathological symptoms. The adrenal weights

TABLE 3. FATTENING LAMBS

Category of Interest	Lot 1 0.66% NaCl	Lot 2 4.8% NaCl	Lot 3 9.4% NaCl	Lot 4 12.8% NaCl
No. of lambs	6	6	6	6
Days on experiment	63	63	63	63
Av. initial wt., lb.	61	60	60	60
Av. daily gain, lb.	0.31	0.32	0.35	0.31
Av. daily feed:				
Basal ration, lb.	3.40	3.28	3.27	3.10
Sodium chloride, lb.	—	0.16	0.33	0.46
Basal ration/100 lbs. gain, lb.	1100	1017	930	1001
Carcass grade:				
No. Choice	4	3	5	3
No. Good	2	3	1	2
No. Utility	—	—	—	1
Dressing percentage	51	49	50	50
Kidney wt., g./kilo body wt.	3.10	3.15	3.46*	3.68**
Adrenal wt., mg./kilo body wt.	71.6	65.6	68.1	73.8
Blood albumin, %	4.93	4.70	4.92	4.97
Hematocrits, %	44	43	45	44

* Significant at the 5% level when compared to Lot 1.
** Significant at the 1% level when compared to Lot 1.

were equal for all groups and equivalent blood albumin and hematocrit levels were noted. The counts of stomach worms showed no differences between groups.

The experiment with fattening steers presented in table 4 essentially confirms the results found with the fattening lambs. The daily gains of the animals were as high for the group receiving the high sodium chloride intakes as for those receiving the basal ration. No difference can be observed between the two groups of steers in dressing percentage; however, there was an advantage for the basal ration in the number of choice carcasses produced. As would be expected, the daily water consumption rose when salt was added to the ration. When calculations were made of the amount of water required per unit of added sodium

chloride intake, it was found that 35 ml. of additional water was required for each additional gram of sodium chloride intake. This is somewhat lower than the 54 ml. that had been previously observed with sheep (Meyer and Weir, 1954).

TABLE 4. FATTENING STEERS

Category of Interest	Lot 1 Basal Ration	Lot 2 9.33% NaCl
Days on experiment	84	84
No. of steers	14	14
Av. initial wt., lb.	815	814
Av. daily gain, lb.	1.56	1.56
Av. daily feed:		
Basal ration, lb.	17.5	16.8
Sodium chloride, lb.	—	1.7
Basal ration/100 lb. gain	1122	1078
Carcass grade		
Choice	9	4
Good	5	10
Dressing percentage	57.1	57.2
Daily water consumption, lb.	110.0	165.9
Av. water intake per gram of added NaCl, ml.	—	35.1

Discussion

These experiments demonstrate that cattle and sheep can be successfully fattened when large amounts of sodium chloride are included in the ration. There is some indication that the higher levels of salt fed to both the sheep and the cattle has decreased the carcass grade.

However, the dressing percentage for all groups of sheep and cattle fed the high salt intakes equalled that of the controls. A longer feeding period would have undoubtedly raised the carcass grade of these animals on the higher salt intakes.

Work with rats (Meyer, 1954) has shown that high sodium chloride intakes decreased daily gains and nitrogen balance. The results with ruminants do not agree with the work with rats in that respect.

In work with high salt intakes, the weighing conditions necessary for properly evaluating the gains are very important. If the animals are weighed immediately after a fill of feed and water, a distorted weight picture occurs. However, if the animals are shrunk on the high salt intake, the extracellular fluid will cause a larger shrink than normal

(Meyer *et al.*, 1955). Therefore, it is important that animals on high salt experiments should be taken off their high-salt rations and shrunk on low sodium chloride rations in order to obtain the correct weight.

Summary

Sodium chloride levels of 0.66, 4.8, 9.4, and 12.8 percent of the ration, or daily intakes of 0.03, 0.16, 0.33, and 0.46 lb., were fed to growing and fattening sheep. These levels of salt had no detectable influence on nitrogen digestibility, nitrogen retention, or total digestible nutrient content of the basal ration. The high salt intakes were not detrimental to average daily gains or efficiency of feed utilization. Carcass grade was maintained until the highest intake was reached. The weights of the kidney increased when the salt intake was 9.4 and 12.8 percent of the ration or a daily intake of 0.33 or 0.46 lb. of salt. The adrenal weights, blood albumin and hematocrits were equivalent for all groups. These high salt intakes had no influence on the number of stomach worms.

A sodium chloride intake of 9.33 percent or a daily intake of 1.7 lb. by fattening steers had no detrimental influence on average daily gains or efficiency of feed utilization. Dressing percentage for the control and the high salt group was the same. There was some decrease in carcass grade with the high salt ration.

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THE THYROID SECRETION RATE OF SHEEP AS AFFECTED BY SEASON, AGE, BREED, PREGNANCY AND LACTATION [1]

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RESEARCH conducted along several different lines indicates that the thyroid secretion plays an important role in the physiology of many of the life processes important to animal production. Various experiments have been conducted in order to study the effect of added thyroactive substances in the rations of animals on growth, fertility and lactation. The data are very conflicting due in part, at least, to the varying levels of hyperthyroidism induced.

This study has been designed to gain more basic information on normal thyroid secretion rates in sheep and the effect of various environmental factors on the secretion rate. A technique is described for measuring the daily thyroid secretion rate in intact, individual sheep which is practical for large animal research.

Development of a Technique for Measuring Thyroid Secretion Rate in the Live Sheep

Wolff (1951) reported the influence of various treatments on the biological output half-life of I^{131} from the thyroid of the rat. The treatments and their respective biological output half-lives were as follows: Untreated, 3.3 and 3.7 days; 0.03 percent propylthiouracil in the diet, 1.6 days; thyrotropin, 8 to 10 hours; hypophysectomy, 24 days; and 15 mg. of d, l-thyroxine daily, 26 days.

Perry (1951) measured the rate of loss of radioactive iodine from the thyroid gland of the rat with an external counter and determined an index of the secretion of thyroid hormone. He checked the validity of this index by the effect of thyrotrophic hormone, goitrogens and thyroxine on the rate of loss of I^{131}. He was able to inhibit markedly the thyroid secretion by the administration of 10 mg. of l-thyroxine and to establish a relationship between lower doses of thyroxine and degree of inhibition of thyroid hormone secretion.

[1] The data reported herein are taken from a thesis presented by the senior author to the School of Graduate Studies, Michigan State College, in partial fulfilment of the requirements of the Ph.D. degree in Animal Husbandry. Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Series No. 1682 N.S.

Procedure

One microcurie of I^{131} per pound of body weight of the sheep was injected subcutaneously on the medial side of the hind leg. All external thyroid counts were taken using a Nuclear Instrument and Chemical Corporation count rate meter with an end-window Geiger-Muller tube. The counts were taken in the area of the neck where the highest count

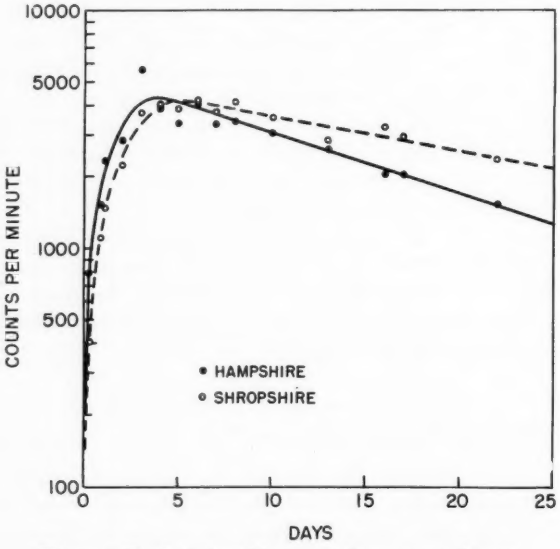

Figure 1. Typical thyroid counts of two control ewes.

could be obtained. The same amount of pressure was used in putting the tube against the neck, insofar as humanly possible.

The background counts were taken on the outside of the foreleg of the sheep and these were subtracted from the external thyroid counts. This procedure corrected approximately for any gamma emissions from I^{131} in the blood stream. At the beginning of each trial the wool was shorn from the neck and foreleg where counts were taken.

A standard source of Co^{60} was used to check the machine at the time the counts were taken on the sheep. This served to eliminate any error due to variable efficiency of the Geiger tube and count rate meter, as each day's readings were corrected to standard. A shield was used on the Geiger tube when all standard, background and external thyroid

counts were taken so only gamma rays were counted. Lastly, the external thyroid counts were corrected to zero time for physical decay of the isotope.

All of the sheep were fed iodized salt, free-choice, in an attempt to insure uniform iodine intake.

Six mature ewes were selected in October, 1951 for preliminary experiments. All of these ewes were injected with I^{131} and corrected

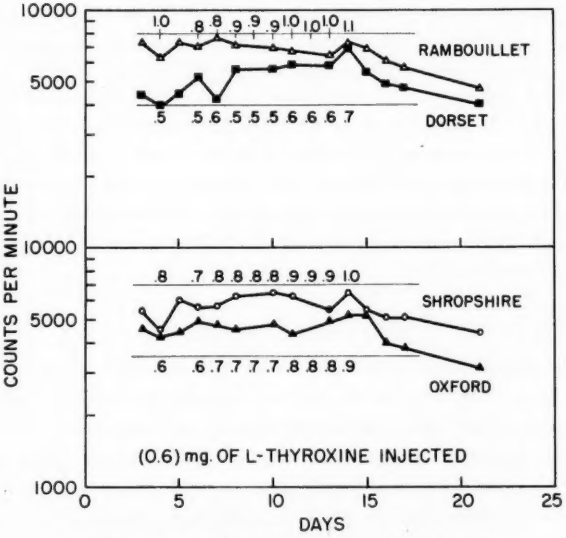

Figure 2. The effect of l-thyroxine injections on external thyroid counts.

counts were obtained in the manner described. The normal uptake and output curves for two of the ewes are illustrated in figure 1. By computation the Hampshire ewe was secreting daily, 2.52 percent of the iodine in the thyroid gland, while the Shropshire ewe was secreting daily, 1.46 percent.

A base reading on the other four ewes was taken on the third day after injection of I^{131}. This was considered zero time and all thyroid counts were corrected for physical decay back to this time. In this study we were concerned with thyroid output and the effect of l-thyroxine injections on thyroid counts. It was expected that the subcutaneous injection of l-thyroxine would exert an effect on the level of

the next days count. At this time the l-thyroxine was used in the form supplied by Glaxo Laboratories, Greenford, Middlesex, England.

The dry thyroxine was weighed on an analytical balance and dissolved in distilled water which was made slightly alkaline with NaOH. Then enough acid (HCl) was added to make the solution slightly cloudy. This solution was then stored in the refrigerator and used within two weeks.

The corrected thyroid counts and the injections of l-thyroxine are plotted on the semi-log scale in figure 2. From these data it is apparent that the injections of l-thyroxine materially affected the slope of the output curve as compared to that portion of the curve when no thyroxine was injected or as compared to the output curves of the two control ewes. It was also apparent from this study that an estimate of normal secretion rate would be very difficult to make and at best would only be a trial-and-error method. This estimate would be based on the assumption that when the count remained at approximately 100 percent of previous count, the injected l-thyroxine was sufficient to replace the normal thyroid secretion.

Predicting Secretion Rate by Linear Regression

In the second trial twelve ewes were injected with I^{131} and 7 days later a base count was taken over the thyroid gland. Four ewes were then injected daily for 3 days with 0.4 mg. of l-thyroxine. Four ewes received l-thyroxine injections of 0.8 mg. daily and the other 4 ewes received 1.2 mg. l-thyroxine daily. Injections of l-thyroxine were made for 3 days in order that the thyroid gland might adjust its secretion to the daily injections. Three days after the base counts were taken of I^{131} in the thyroid, a second external count was taken and corrected for physical decay. The percentage of previous count was then determined for each ewe.

These percentages of previous count are plotted in figure 3. It was unfortunate that all of the selected levels of l-thyroxine injections yielded percentages of previous count in excess of 100 percent. Therefore, any predicting equation for estimating daily thyroid secretion rate would be meaningless as demonstrated by the regression line drawn from the data. However, the data did demonstrate that percentages of previous count could be obtained that were greater than 100 percent. In other words, the thyroid was accumulating and storing additional I^{131} from the blood stream from 7 to 10 days after injection of I^{131}.

Due to the fact that the design of this trial would only give us an average secretion rate for a group of sheep a variation on this method was employed. An attempt was made to extrapolate the thyroid secretion rate from varying levels of l-thyroxine injection and a series of external counts on individual lambs.

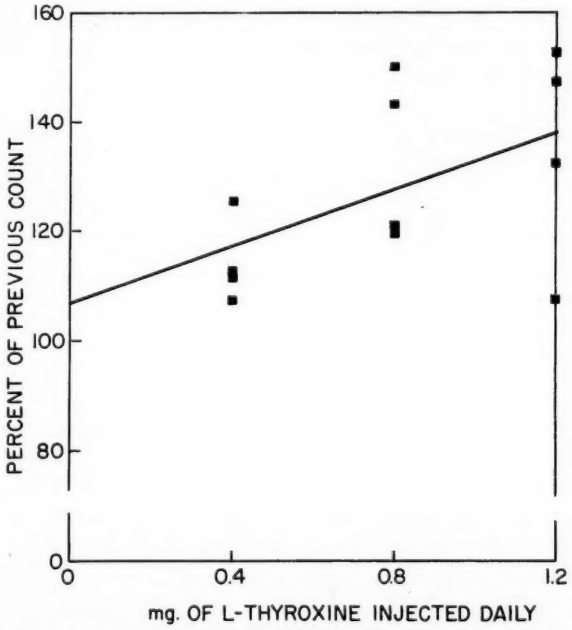

Figure 3. The effect of thyroxine injections on percentage of previous count.

Previous to the start of this trial the stock supply of l-thyroxine was purified by successive recrystallization. The activity of the l-thyroxine increased, necessitating a lower level of thyroxine injection if reliable estimates of secretion rate were to be obtained.

Four lambs were used in this trial. The I^{131} injection and external counts were conducted in the same manner as in the previous trials. At 7 (zero time) and 10 days, post injection of I^{131}, base counts were taken over the thyroid gland. Four more counts were taken at 3-day intervals. For the first 3-day period following the determination of the base counts no thyroxine was injected, but a thyroid count was taken. For

the second 3-day period each lamb received a 0.1 mg. injection of l-thyroxine daily and another external count was taken. During the next 3-day period 0.25 mg. of thyroxine, and then for the last 3-day period 0.5 mg. of thyroxine was injected daily.

These counts were then corrected for background and physical decay, to zero time, and the percentage of previous count was computed. As the quantity of l-thyroxine injected daily increased, the percentage of previous count increased, which indicated that the thyroid itself was secreting less thyroxine.

TABLE 1. CORRELATION AND REGRESSION COEFFICIENTS AND ESTIMATED DAILY L-THYROXINE SECRETION

Item	D.F.	r	Level of Significance	b	Estimated Daily Secretion
					mg.
Crossbred 52	3	.992	.01	.421	.37
Crossbred 69	3	.981	.05	.619	.39
Shropshire	2	.983	.15	.454	.23
Hampshire	3	.837	.10	.424	.57
Intra-lamb	11	.928	.01	.485	.39

The data were analyzed statistically and the correlations between percentage of previous count and mg. of l-thyroxine were obtained for each individual lamb as well as on an intra-lamb basis. The regression coefficients were calculated and the predicting equation ($y=a+bx$) was used to estimate the daily thyroxine secretion rate of each lamb. It was assumed that where the predicting line crossed 100 percent of previous count, this represented the daily secretion rate. The correlation and regression coefficients and the estimated secretion rates are presented in table 1 and the predicting lines are shown graphically in figure 4.

Discussion

In using the technique of injecting varying levels of thyroxine in individual sheep and obtaining the percentage of previous count, several problems arise.

First of all an attempt has been made to use tracer doses of I^{131} that would not impair the thyroid function of the sheep. Therefore it was found that only 4 and at the most 5 readings of the thyroid count could be taken with a G.M. tube when 3 days elapsed between counts, before the external thyroid counts had dropped to a level 2 to 3 times the

background count. From the standpoint of statistics, the use of the predicting equation with only 4 items was hazardous. To further complicate the picture, it was observed that once a reading of around 100 percent of previous count was obtained the injection of a higher level of l-thyroxine resulted in a very unreliable percentage of previous count. It

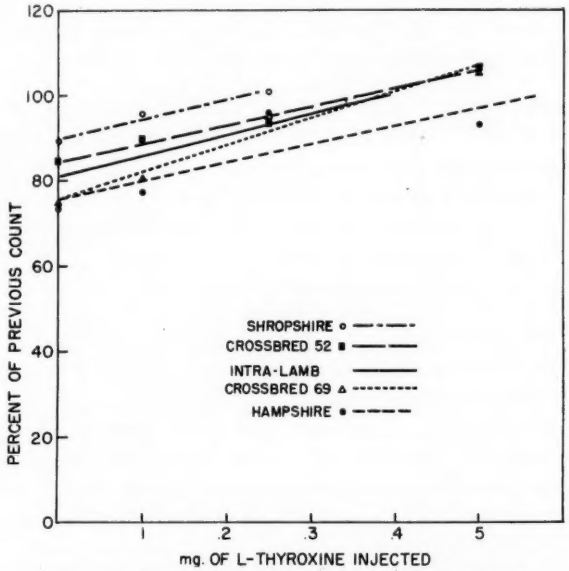

Figure 4. Extrapolation of daily secretion rate from predicting equation.

could be higher, about the same or much lower than the figure obtained for percentage of previous count at the lower level of thyroxine injection. Therefore, in this study after a reading of about 100 percent previous count was obtained any further readings obtained using a higher level of thyroxine injection were discarded. Not having any previous data on thyroxine secretion rate in sheep under various environmental conditions the level of thyroxine injections could only be estimated. This explains why the number of items used in the predicting equation of thyroxine secretion in individual sheep varied between 3 and 5.

The intra-lamb correlation and regression coefficients are the most accurate estimate for an entire group of sheep. However, in order to

study the differences in thyroxine secretion between different groups of sheep, it was necessary to obtain estimates of individual secretion, as the variations between individuals is a necessary statistic in determining significant differences between groups.

With a full realization of the statistical shortcomings of this procedure, the following estimates of thyroxine secretion on sheep are reported. As a measure of significance of the estimated secretion rate, the probability that the regression coefficient was significantly different from zero is reported along with the estimated secretion rate.

Application of the Extrapolation Technique to the Study of Differences in Thyroid Secretion Rate of Sheep

Twenty four ewes were selected at the beginning of the year to be used in studying the differences in thyroid secretion rate due to season, age, breed, pregnancy and lactation. They were divided into 4 groups with 6 animals in each group as follows:

6 open 2-year old Shropshire ewes, av. body wt. 114 lb.
6 bred 2-year old Shropshire ewes, av. body wt. 117 lb.
6 bred 4-year old Shropshire ewes, av. body wt. 139 lb.
6 bred 2-year old Hampshire ewes, av. body wt. 141 lb.

Due to deaths of lambs or ewes during the year, the number of sheep in each group was not maintained. Half of the open ewes were accidentally bred and, therefore, only 3 ewes remained in the open group for determinations in January and March. When these were lambed, the lambs were immediately removed and they were considered as dry while the other ewes were milking.

The entire group of ewes was handled in one flock under similar feeding conditions and iodized salt was fed free-choice. All of the ewes were sheared in February.

When the external thyroid counts were taken, the sheep were selected in the same order each time and the readings were taken at the same hours of the day. Thus, all readings were 72 hours apart.

Results and Interpretation

Seasonal Differences in Secretion Rate The group of 2-year-old open Shropshire ewes was used to study the effect of season of the year upon daily thyroid secretion rate to eliminate any differences which may be due to pregnancy and lactation.

The estimated individual thyroid secretion rates by months are given in table 2. The analysis of variance was used and the "F" value obtained indicated a significant difference between at least two of the means. The

TABLE 2. MILLIGRAMS OF L-THYROXINE SECRETED DAILY BY OPEN TWO-YEAR OLD SHROPSHIRE EWES DURING VARIOUS MONTHS OF THE YEAR

	January		March		May		July		September		December	
Ewes	Daily Secretion	P[1]	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P
	mg.		mg.		mg.		mg.		mg.		mg.	
923	.18	.25	.15	.01			.03	.05	.14	.05	.15	.05
916			.25	.05	.19	.05			.13	.05		
940	.12	.05	.20	.05	.16	.255	.07	.06	.19	.05	.16	.10
929					.09	.25	.03	.01	.12	.05	.17	.05
952					.23	.10	.4	.30	.12	.05	.15	.05
Mean	.15		.20		.17		.04**		.14		.16	

[1] Probability that the regression coefficient is significantly different from 0.
** July secretion significantly lower than all other months at 1% level.

standard deviation of the means and standard deviation of the difference between the means was computed. The "Student t" table was then used to test for the significance of differences between the means.

The daily thyroid secretion rate in July (0.04 mg.) was significantly lower ($P<.01$) than the estimated secretion rates in any other month. Also in September the secretion rate (0.14 mg.) was significantly lower ($P<.05$) than in March (0.20 mg.). All of the sheep were sheared in February which may account for the increase in secretion rate during March (0.20 mg.) as compared to January (0.15 mg.).

In line with these results, Reineke and Turner (1945) observed a seasonal trend in thyroid secretion rate of young chicks, the rate in spring and summer being only about one-half the winter level.

Brody and Procter (1932) reported a seasonal energy metabolism rhythm in sheep which they believed to be associated with the seasonal sex rhythm.

Berliner and Warbritton (1937), and Bogart and Mayer (1946) concluded that high summer temperatures were a major factor in the production of summer sterility in rams and, also, that the thyroid was of major importance in the reproductive physiology of the ram.

The Effect of Pregnancy and Lactation on Thyroid Secretion Rate. Comparisons between pregnant and non pregnant ewes during the months of January, March, and December, indicate that there was no significant difference in the daily thyroid secretion rate. These comparisons were made between open 2-year old Shropshire ewes and bred 2-year old Shropshire ewes by months. Therefore, any effect of age or breed of sheep or month of the year was removed.

The individual thyroid secretion rates for two-year old bred and lactating Shropshire ewes are given in table 3.

During March, there was a significant difference at the one percent level between open 2-year old Shropshire ewes (0.20 mg.) and lactating 2-year old Shropshire ewes (0.32 mg.). Also, during March there was a significant difference at the 5 percent level between pregnant 2-year old Shropshires (0.24 mg.) and the lactating 2-year old Shropshires.

In May, open 2-year old Shropshire ewes (0.17 mg.) secreted significantly less thyroxine daily (P less than .05) than lactating 2-year old Shropshires (0.24 mg.).

Comparisons can also be made between pregnant and lactating ewes in the month of March for 4-year old Shropshire ewes and 2-year old Hampshires although the numbers of sheep involved are not large enough to lend significance to these differences. These data are given in table 4.

TABLE 3. AMOUNTS OF L-THYROXINE SECRETED DAILY BY PREGNANT AND LACTATING TWO-YEAR OLD SHROPSHIRE EWES.

	January		March				May		July		September		December	
	Bred		Bred		Lactating		Lactating		Dry		Open		Bred	
Ewes	Daily Secretion	P[1]	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P
	mg.		mg.		mg.		mg.		mg.		mg.		mg.	
929	.14	.25	.25	.15										
952	.19	.07	.29	.05										
902			.20	.10			.25	.10	.06	.25	.12	.01	.18	.05
919	.21	.40	.24	.05			.23	.01	.03	.01	.12	.01	.14	.10
911	.12	.25	.24	.05			.25	.10	.03	.40	.09	.15	.15	.01
927	.17	.01			.27	.10								
903	.15	.05			.35	.05	.19	.05	.06	.25	.10	.05		
924					.27	.05	.22	.01	.03	.01	.11	.01	.13	.15
905	.21	.20			.41	.07	.32	.10	.03	.01	.12	.01	.16	.05
Mean	.17		.24		.32		.24		.04		.11		.15	

[1] Probability that the regression coefficient is significantly different from 0.

TABLE 4. MILLIGRAMS OF L–THYROXINE SECRETED DAILY BY PREGNANT AND LACTATING 4-YEAR OLD SHROPSHIRE AND 2-YEAR OLD HAMPSHIRE EWES

	January		March				May		July		September		December	
	Bred		Bred		Lactating		Lactating		Dry		Open		Bred	
Ewes	Daily Secretion	P[1]	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P	Daily Secretion	P
	mg.		mg.		mg.		mg.		mg.		mg.		mg.	
513[2]	.15	.05												
552[2]			.15	.15			.09	.35	.03	.01	.06	.05	.09	.10
517[2]	.09	.05	.15	.05			.12	.05	.03	.01	.09	.05	.11	.10
546[2]			.16	.15			.10	.50	.03	.35	.14	.20	.11	.01
558[2]					.18	.25			.01	.20	.14	.05	.10	.01
541[2]	.11	.10			.21	.06								
502[2]	.16	.20			.25	.45	.10	.15	.02	.01	.04	.01	.09	.05
Mean[2]	.13		.15		.21		.10		.02		.09		.10	
831[3]	.29	.01	.24	.15					.10	.10	.10	.15	.14	.05
816[3]	.27	.07	.33	.10										
809[3]	.30	.10	.27	.01			.23	.05	.03	.15	.15	.05	.20	.01
823[3]	.28	.10	.21	.10			.27	.01	.03	.25	.19	.06	.17	.05
832[3]	.25	.01	.18	.07			.21	.05	.07	.20	.18	.01	.17	.06
834[3]					.33	.05	.24	.05	.03	.25	.16	.05	.19	.05
Mean[3]	.28		.24		.33		.24		.05		.16		.18	

[1] Probability that the regression coefficient is significantly different from 0.
[2] 4-year old Shropshires.
[3] 2-year old Hampshires.

Bred 4-year old Shropshires secreted 0.15 mg. of thyroxine daily in March as compared to 0.21 mg. for lactating 4-year old Shropshires. Bred 2-year old Hampshires secreted 0.24 mg. daily as compared to 0.33 mg. for the one lactating Hampshire ewe.

Very few data appear in the literature in regard to this problem. Monroe and Turner (1948) reported no significant differences from normal in pregnant and lactating rats. Rugh (1951) observed that the mouse thyroid was protected from radiation damage by the process of lactation indicating there was a greater output of radioactive iodine from the thyroid gland.

It was shown by Graham (1934a, 1934b) and others that thyroid feeding or thyroxine injection increased both the milk and fat yield of dairy cows. Similar effects were obtained by feeding thyroactive iodinated casein (Reineke and Turner, 1942). The increase in milk yield obtained by feeding thyroidal materials has amounted to as much as 50 percent, (Reineke, 1943; Archibald, 1945).

The Effect of Age on Thyroid Secretion Rate. In studying differences in thyroid secretion rate due to age, the 2-year old Shropshire ewes were compared to the 4-year old Shropshire ewes. During July and September all of the 2-year old Shropshire ewes were used for the comparison but during the other months only the bred or lactating 2-year olds were used.

For convenience, the average secretion rates and the probability that there was a significant difference are given in table 5. There was a significant difference due to age except during the months of July and September and in all cases the older ewes were secreting thyroxine at a lower level than the younger ewes.

Turner (1948) observed a pronounced decline in the thyroid secretion rate of older hens. By use of the protein-bound iodine technique, Long *et al.* (1951) have observed differences due to age in cattle.

From these results, it would appear that age must be taken into consideration when studying levels of thyroid secretion or when thyroactive substances are being fed.

The Effect of Breed on Thyroid Secretion Rate. Hampshire and Shropshire 2-year old ewes were used in this study in an attempt to determine breed difference in daily thyroid secretion rate. Data on the Shropshire ewes are presented in table 3 and data on Hampshires in table 4.

During the month of January, Hampshires secreted significantly more ($P<.01$) thyroxine daily (0.28 mg.) than Shropshires (0.17 mg.).

In December, Hampshires (0.18 mg.) also secreted more thyroxine ($P<05$) than Shropshires (0.15 mg.). In September (when Hampshires secreted 0.16 mg. and Shropshires 0.11 mg.), the difference only reached the 10 percent level of significance. During March, May and July, the average amount of thyroxine secreted daily by the two breeds was very similar.

From these data, it would appear that differences between these two breeds is not as large as the differences in thyroid secretion due to season, age and lactation.

TABLE 5. AVERAGE THYROID SECRETION RATES BY MONTHS

Comparison	2–year old	4–year old	P.
	mg.	mg.	
January (bred)	.17	.13	.05
March (bred)	.24	.15	.05
March (lactating)	.32	.21	.01
May (lactating)	.24	.10	.01
July (dry)	.04	.02	
September (open)	.12	.09	.10
December (bred)	.15	.10	.01

Berliner and Warbritton (1937) observed that Shropshire rams were more subject to summer sterility than Hampshire rams, and the thyroids of Hampshires were believed to be more active. They also alleviated summer sterility by the administration of thyroxine.

Mixner and Upp (1947) reported that "double cross" hybrid chicks had a considerably higher thyroid secretion rate than either "single cross" or ordinary breeds of chicks.

Hurst and Turner (1948) observed differences in secretion rate in three strains of mice. Long *et al.* (1951) observed differences in protein bound iodine levels of blood serum between breeds of dairy and beef cattle.

Discussion. The method described in this report offers for the first time a means of estimating in absolute terms the thyroid secretion rate of the intact living sheep. While the method of external thyroid counting employed is admittedly somewhat crude, the procedure has the advantage of measuring changes in thyroid activity at its source, i.e. in the thyroid gland. Although changes observed by this method would be expected to be similar to those indicated by the method for protein bound iodine (Long *et al.*, 1951) this would not necessarily be true. The present method measures thyroid output directly whereas blood

PBI values represent the balance between thyroid output, distribution to the tissues, metabolism and excretion.

As shown in the present study, the thyroid secretion of the sheep varies markedly. Whether the approximately 4-fold reduction in summer is due principally to the depressing effect of high temperature, increased light exposure or a combination of both cannot be determined from the information available because these factors could not be controlled separately under our conditions. In rats, both high temperature (Dempsey and Astwood, 1943) and continuous light (Meites and Puntriano, 1951) depress thyroid function.

The increase in thyroid secretion during lactation is of special interest in view of the many earlier reports that thyroprotein feeding increases lactation.

Further research is needed to refine the extrapolation technique so that it can be applied to the study of variations of thyroid secretion rate in individual animals. The development of an adequate statistical treatment is also needed to determine the confidence limits of such individual measurements.

In our opinion, this method provides a new and more effective approach to the study of the factors influencing thyroid function. It should also make possible more precise studies on the influence of the normal thyroid status on productive processes.

Summary

A technique is described for measuring the daily thyroid secretion rate in intact, individual sheep.

From 24 experimental ewes divided into four groups and using the extrapolation technique, the following results were observed.

The season of the year has a great effect upon thyroid secretion in sheep. In July a daily thyroxine secretion of 0.04 mg. was significantly lower than at any other month readings were taken. The next lowest secretion was observed in September (0.14 mg.). There was a significant difference (P less than .05) between September and March (0.20 mg.).

Pregnancy in sheep does not cause a significant difference in thyroid secretion.

Lactation creates increased demands upon the thyroid gland as lactating 2-year old ewes had a higher thyroid output than either bred or open 2-year old ewes.

Four-year old ewes secreted significantly less thyroxine daily than 2-year old ewes during January, March, May and December. However, differences during July and September were not significantly different.

Significant breed differences in thyroid secretion rate between Hampshire and Shropshire ewes were observed only in January and December.

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TOXICITY OF SOME ARSENICALS FED TO GROWING-FATTENING LAMBS [1, 2, 3, 4]

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PREVIOUS work by Bucy *et al.* (1954) has shown that 3-nitro-4-hydroxyphenylarsonic acid, arsanilic acid, and potassium arsenite fed to growing-fattening lambs in practical rations at levels up to 0.024 percent of the total ration will not affect growth or cause pathological damage. Frost (1953) reported 3-nitro-4-hydroxyphenylarsonic acid was much more toxic to chicks and ducks than arsanilic acid. It seems to be generally accepted that 3-nitro-4-hydroxyphenylarsonic acid is more toxic to swine than arsanilic acid.

Two experiments were conducted to study the toxic levels, symptoms of toxicity, and some physiological effects of these arsenicals when fed to sheep.

Experiment 1

Procedure

Twelve western feeder lambs ranging in weight from 54 to 77 lb. were selected for the test. All lambs were drenched with one ounce of phenothiazine one week before the trial began, and all appeared to be in a healthy, thriving condition.

The basal ration consisted of 50 percent ground alfalfa hay, 45 percent ground yellow corn, and 5 percent of 44 percent soybean meal by weight. 3-nitro-4-hydroxyphenylarsonic acid was fed at 0.05, 0.1, 0.2, and 0.4 percent of the total ration, and arsanilic acid and potassium

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arsenite were fed at levels to supply equal amounts of arsenic.[8] The arsenicals were premixed with the soybean meal before mixing with the ground hay and ground corn in a twin spiral mixer.

The lambs were individually self-fed in 4 by 4 foot stalls, each lamb being fed a different one of the 4 levels of 3 compounds. Water was available at all times. Wood shavings were used for bedding. The lambs were fed the experimental rations for 56 days and were weighed weekly.

Arsenic determinations were made on liver, kidney, and muscle of all lambs using the Evans and Bandemer method (1954).

Representative sections of the liver and kidney of each animal were fixed in Bouin's solution, embedded in paraffin, sectioned at 8 microns, and stained with hematoxylin and eosin, basic fuchsin and acidic potassium ferrocyanide (Lillie, 1948) for histological examination.

Results and Discussion

The growth data were analyzed statistically and there was no significant difference due to arsenic compounds or level of arsenic fed (table 1). The gain or loss of weight appeared to be more closely correlated to feed consumption than to amount of arsenic consumed. Lamb No. 1506 (0.2 percent 3-nitro-4-hydroxyphenylarsonic acid) ate 85 lb. of feed and gained 20 lb. in the first 5 weeks of the test. During the sixth week, 0.4 percent 3-nitro-4-hydroxyphenylarsonic acid feed was substituted for the 0.2 percent 3-nitro-4-hydroxyphenylarsonic acid feed; the feed consumption decreased, and the lamb lost weight during the remainder of the test.

The arsanilic acid rations were not eaten as well as the 3-nitro-4-hydroxyphenylarsonic acid rations. Lamb No. 1513 (0.4 percent arsanilic) did not eat well and convulsions were observed during the fourth week of the test. The lamb was observed to fall down with its entire body shaking and becoming rigid with the head drawn backward. The convulsions usually lasted less than one minute after which the lamb would stand and appear normal. However, after a few days the lamb was not able to rise without aid, but could stand when on its feet. When it became apparent the lamb would not recover, it was used for tissue studies; no gross tissue changes were observed. Lamb No. 1503 (0.2 percent arsanilic) exhibited convulsions similar to Lamb No. 1513 during the eighth week. This lamb had eaten more feed and was in a stronger, more active condition. Convulsions were initiated in both when

[8] 3-nitro-4-hydroxyphenylarsonic acid at 0.05 percent is equivalent on the basis of arsenic content to 0.041 percent of arsanilic acid and 0.028 percent of potassium arsenite in the total ration.

TABLE 1. DATA ON INDIVIDUAL LAMBS IN EXPERIMENT 1 SELF-FED RATIONS CONTAINING RELATIVELY HIGH LEVELS OF ARSENIC

Lamb No.	Arsenical	Level	Arsenic Consumed	Initial Weight	Feed Consumed	Weight Gain	Arsenic Content (ppm)		
							Liver	Kidney	Muscle
		%	gm.	lb.	lb.	lb.			
1516	3-nitro[1]	0.05	5.9	77	91	7	6.8	7.8	0.62
1505	3-nitro	0.1	16.2	67	106	9	13.3	7.9	1.40
1506	3-nitro	0.2	34.8	60	117	14	13.3	9.8	1.30
1515	3-nitro	0.4	37.4	66	72	9	9.3	10.5	0.44
1502	Arsanilic A.	0.05	12.4	70	135	10	12.3	13.5	1.70
1509	Arsanilic A.	0.1	8.7	54	68	3	9.3	7.5	0.30
1503	Arsanilic A.	0.2	15.3	74	57	−17	12.3	8.4	2.30
1513	Arsanilic A.	0.4	12.0	66	23	−19	8.3	7.1	....
1514	K-Arsenite	0.05	6.4	61	98	11	10.0	7.7	2.20
1438	K-Arsenite	0.1	14.8	64	107	9	9.0	9.8	3.50
1501	K-Arsenite	0.2	7.3	66	28	−18	12.3	13.5	1.70
1504	K-Arsenite	0.4	3.1	69	27	−22	8.5	5.9	2.20

[1] 3-nitro-4-hydroxyphenylarsonic acid.

moved about or excited. Figures 1, 2 and 3 depict Lamb No. 1503 just before it was killed on the last day of the experiment.

The potassium arsenite rations were eaten in smallest amount and the 0.4 percent ration was refused after the third week. After two weeks of feed refusal, the 0.4 percent potassium arsenite ration was replaced with non-supplemented feed to see if the lamb would eat. The lamb ate

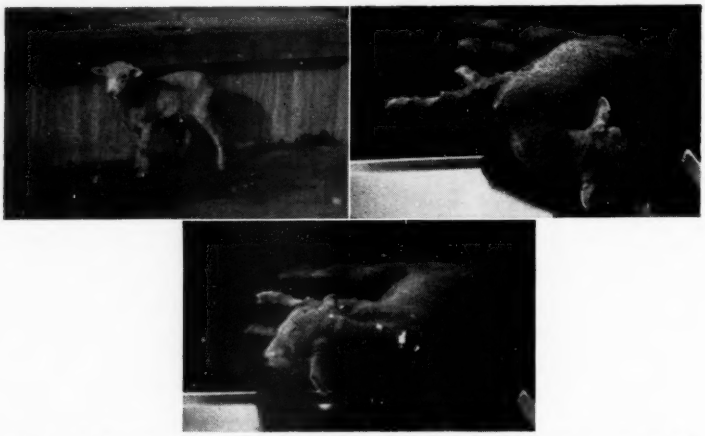

Figure 1. Lamb No. 1503 (0.2 percent arsanilic acid) at the end of the 56-day feeding trial. It had been having convulsions for 3 days but appeared normal except during a convulsion. The next 2 pictures are of the same lamb a few minutes later.

Figure 2. Lamb down and unable to rise during a convulsion. Note the rigid legs and drawn neck.

Figure 3. A few seconds after Figure 2, the lamb was trying to rise but was still unable to get onto its feet.

the basal feed well and gained some weight, but during the eighth week, when the supplemented feed was again given, the lamb refused to eat and lost weight during that week.

No other toxicity symptom was noted during the experiment even though the lambs on the 0.2 and 0.4 percent 3-nitro-4-hydroxyphenylarsonic acid rations consumed about 3 times as much arsenic as the lambs on the 0.2 and 0.4 percent arsanilic acid rations.

Blood samples were taken at the end of 3 weeks and the prothrombin times determined by Quick's method. There was no significant difference between treatments or between the treated and control lambs.

Histological examination of the liver and kidney showed that pathological changes occurred in these organs following the feeding of arsenicals. There was cloudy swelling in the liver cells and a positive ionic iron (ferrous) reaction in the Kupffer cells of those animals which received more than 0.05 percent 3-nitro-4-hydroxyphenylarsonic acid in the diet. All levels of arsanilic acid produced cloudy swelling in the liver, while those animals on high levels had livers which contained many phagocytes, areas of cirrhosis, and foci of polymorphonuclear cells. Potassium arsenite induced similar morphological hepatic changes, but the changes were more pronounced.

Kidney damage noted consisted of albumin casts in the collecting ducts, a tannish intravscular pigment in the medullary portion, pigment in the veins and small blood vessels in the renal medulla, intracellular ionic iron in the cells of the tubules, casts in the blood vessels, and necrotic debris in the tubules in all treatments and at all levels. None of these morphological changes was observed in the liver or kidneys of the control lambs. The damage was not as great in the 3-nitro-4-hydroxyphenylarsonic-acid-fed lambs. Figures 4, 5 and 6 show kidney damage and deposition of pigment within the cells of the tubules. There was no apparent difference between the adrenal glands of these lambs and the controls.

The Evans and Bandemer method of arsenic determination (1954) was used to determine the arsenic content of the liver, kidney, and muscle tissues (table 1). There was a highly significant difference between the amount of arsenic in all of these tissues and the tissues of control and treated lambs of previous trials (table 2). In earlier work at much lower arsenic levels, Bucy *et al.* (1954), studied the disappearance of arsenic from the tissues of lambs killed at intervals after being taken off the arsenic-containing rations. There were significant differences between the arsenic contents of the livers of control and treated lambs, between the livers of treated lambs on different levels of arsenic, between the livers of treated lambs on different killing dates, and between the lambs receiving the organic arsenicals and those fed the potassium arsenite. There was a significant difference in arsenic content between the kidneys of the treated and control groups. The arsenic content of the kidneys decreased rapidly after the lambs were taken off the arsenicals, and the killing date appeared to influence the arsenic content of the kidney more than the level or compound used. The difference between the arsenic contents of the muscle of control and of treated lambs approached the 1 percent level of significance.

There appeared to be no difference between different compounds or different levels. However, the killing date appeared to influence the arsenic content of the gluteus maximus muscle as the difference approached the 5 percent level.

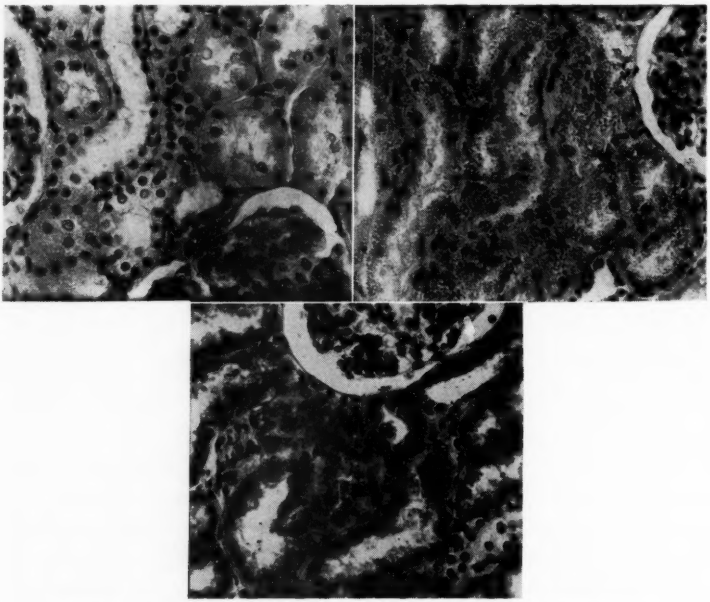

Figure 4. Section of kidney from control lamb (hematoxylin-eosin). X460

Figure 5. Kidney of lamb fed 0.2% K-Arsenite (hematoxylin-eosin). Note breakdown of tubular membrane, slight fatty infiltration, deposition of pigment (brown) within the tubular cells. X460

Figure 6. Kidney of same lamb as in Figure 6 (basic fuchsin and acidic potassium ferrocyanide). Note dark pigment granules (green) indicating ferrous iron and the presence of hemosiderins. Bowman's capsule and glomerular apparatus are normal. X460.

The development of convulsions as a result of arsenic consumption may be a result of impaired carbohydrate utilization. Peters (1953) has pointed out that arsenic poisoning results in increased blood pyruvate levels, indicating inhibition of pyruvate oxidation. Arsenic can combine with dithiol linkages, one of which is present in lipothiamide pyrophosphate, thus providing a reasonable basis for explaining failure

TABLE 2. ANALYSES OF ARSENIC IN TISSUES OF LAMBS FROM PREVIOUS TRIALS, BUCY *et al.* (1954)

Treatment	Date[1] Killed	Arsenic (ppm Fresh Tissue) Liver	Kidney	Muscle
Basal		.05	.09	.05
		.21	.13	.14
		.18	.11	.10
		.21	.19	.10
		.05	.10	.09
		.18	.26	.19
K-Arsenite, %				
0.002	1	.16	.23	.48
	2	.22	.18	.33
	3	.28	.13	.26
0.004	1	.18	.26	.24
	2	.25	.13	.20
	3	.25	.24	—
0.006	1	.98	.46	.26
	2	.71	.23	.56
	3	.23	.25	.14
Arsanilic acid, %				
0.002	1	.93	.74	.23
	2	.54	.20	.14
	3	.21	.31	.11
0.004	1	1.13	.51	.45
	2	.31	.23	.09
	3	.23	.24	.15
0.006	1	—	.74	.44
	2	.84	.56	.13
	3	.49	.30	.11
3-nitro-4-hydroxyphenyl-arsonic acid, %				
0.002	1	.45	.76	.94
	2	.26	.40	.11
	3	—	.15	.10
0.004	1	.36	.60	.29
	2	.21	.41	.14
	3	.26	.26	.21
0.006	1	.41	.59	.31
	2	.69	.36	.25
	3	.30	.21	.14

[1] All No. 1 lambs killed while on arsenical ration; all No. 2 lambs killed after 3 days on basal ration; and, all No. 3 lambs killed after 6 days on basal ration.

of pyruvate oxidation and development of functional lesions of nerve tissue which depends largely on carbohydrate for energy.

Nicholson (1945) reported post-mortem appearances in arsenic poisoning to be red inflammation of the alimentary tract, with ecchymoses and extravasation of blood, mucus membranes swollen, and fatty degeneration of liver, kidney and heart.

Von Glahn *et al.* (1938) reported arsenates caused necroses in the livers of rabbits. The healing of these necroses resulted in cirrhosis.

Experiment 2

Procedure

Nine mature ewes were used in the second experiment planned to confirm the findings in Experiment 1. The procedure followed was as in Experiment 1 with these exceptions: mature ewes were used that ranged in weight from 125 to 192 lb., the 0.05 percent level of arsenical supplementation was not used, prothrombin times were not run, blood studies were made at time of slaughter, and urine samples were examined for bile pigments and ionic iron. The arsenicals were weighed and premixed as in Experiment 1.

Qualitative tests for the detection of urinary bile pigments (Smith's, Gmelin's, Schlesinger's) and for the detection of urinary hemosiderins (Rous's) were run on the urine of each ewe. The serum bilirubin content was determined (Todd and Sanford, 1948). Histological studies were the same as in Experiment 1.

Results and Discussion

The ewes were fed the experimental rations for 56 days. The ewes fed 3-nitro-4-hydroxyphenylarsonic acid ate much better than the ewes receiving the other arsenicals and maintained their weight better even though they consumed much more arsenic (table 2). Ewe No. 9 (0.4 percent potassium arsenite) refused to eat the supplemented feed and died on the 28th day of the test. She was in very good condition when the test began and lost 43 lb. of weight during the 4 weeks but was not in emaciated condition at the time of death. The diagnostic clinic of the College of Veterinary Medicine was unable to determine the cause of death. Ewe No. 8 (0.2 percent potassium arsenite) ate only nine lb. of feed during the 56 days and gave birth to a lamb on the 16th day of the experiment. She lost 82 lb. during the test, but showed no toxicity symptom. Ewe No. 6 (0.4 percent arsanilic acid) began to show

TABLE 3. DATA ON INDIVIDUAL EWES IN EXPERIMENT 2 SELF-FED RATIONS CONTAINING RELATIVELY HIGH LEVELS OF ARSENIC

Ewe No.	Arsenical	Level	Arsenic Consumed	Initial Weight	Feed Consumed	Weight Gain	Arsenic Content (ppm)		
							Liver	Kidney	Muscle
		%	gm.	lb.	lb.	lb.			
1	3-nitro[1]	0.1	31.5	125	242	18	4.75	4.13	0.40
2	3-nitro	0.2	56.2	163	216	0	8.00	5.50	0.48
3	3-nitro	0.4	60.3	185	116	—10	6.75	10.50	0.60
4	Arsanilic	0.1	17.7	130	136	—27	13.75	2.50	0.65
5	Arsanilic	0.2	20.3	133	78	—27	12.75	7.75	1.35
6	Arsanilic	0.4	10.2	164	20	—49	11.50	11.00	2.65
7	K-Arsenite	0.1	6.0	137	47	—46	8.25	9.00	1.35
8	K-Arsenite	0.2	2.4	162	9	—82	10.50	12.00	3.05
9	K-Arsenite	0.4	0	192	0	—43	.....	.....	....

[1] 3-nitro-4-hydroxyphenylarsonic acid.

signs of weakness on the 34th day of the test and was unable to rise or stand without aid on the 36th day and died on the 39th day. She did not have convulsions as did the lamb on this level of arsanilic acid in Experiment 1. No other arsenic toxicity symptom was noted during the test.

Chemical analysis of the liver, kidney, and muscle showed about the same pattern as Experiment 1 (table 3).

Histological changes in the liver and kidney were similar to those found in Experiment 1, but the damage was much more extensive. Results of the tests for urinary (high) and serum (high) bile pigments and urinary hemosiderin (high), the altered red blood cell count (low), hemoglobin (low), and white blood cell count (low), plus the morphological changes in the liver and kidneys, indicate a hemolytic condition in lambs which are fed arsenicals.

Summary

Individual self-feeding was used to determine toxic levels and symptoms of toxicity of three different arsenicals in sheep. Practical Corn Belt growing-fattening rations made up of 50 percent alfalfa hay, 45 percent corn, and 5 percent soybean meal were used.

Potassium arsenite, arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid were used to supply equal amounts of arsenic at levels of 0.05, 0.1, 0.2, and 0.4 percent of the total ration based on the 3-nitro-4-hydroxyphenylarsonic acid. 3-nitro-4-hydroxyphenylarsonic acid rations seemed the most palatable, especially at the higher levels, and the potassium arsenite rations were consumed in the least amounts.

Lambs on the 0.2 and 0.4 percent arsanilic acid rations became convulsive in Experiment 1 and the ewe on the 0.4 percent ration in Experiment 2 died before the test ended.

The levels of arsenicals fed in these experiments did not affect the prothrombin time. However, pathological changes in the livers and kidneys decreased red blood cell count and decreased hemoglobin levels, high levels of serum and urinary bile pigments, and the presence of hemosiderins in the Kupffer cells, the renal tubule cells, and in the urine, indicate a severe hemolytic condition.

The arsenic content of the liver, kidney, and muscle was significantly increased by feeding arsenicals.

It appears that 3-nitro-4-hydroxyphenylarsonic acid was not as toxic to sheep as arsanilic acid or potassium arsenite. Potassium arsenite seemed very unpalatable, and when it was fed at the 0.2 percent level

these sheep went off feed. These arsenicals may cause severe pathological damage without outward toxicity symptoms.

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A PRELIMINARY STUDY OF SELENIZED WOOL

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A PRELIMINARY study of selenized wool was initiated in an attempt to find the presence of selenium in the body and fleece of the sheep. Several workers have shown that selenium in the bodies of animals can be determined chemically by testing the blood, body tissues, and organs for selenium or by waiting for the characteristic syndrome of selenium poisoning. As selenium indicator-plants can not always be found on selenium ranges, it would be helpful to the sheepman who was suspicious of his range if a test of the chemical or physical properties of the wool would indicate the presence or absence of selenium. Another point of interest to the wool man is that wool from selenized areas seems to have relatively harsh handle, not soft and pliable, which would decrease the value of the wool.

Selenium is an element with an atomic weight of 78.9, and was discovered in 1817 by Berselius who named it in tribute to Selene, the Greek Goddess of the moon. Its intimate association with sulfur has long been recognized, and it possesses many of the physical and chemical properties of sulfur, tellurium and arsenic. This element has been found in various geological formations in most of the western states of North America and Mexico, and the seleniferous areas can be plotted by the use of the Beath indicator plants which survive only when selenium is in the soil. The plants are divided into two groups (Trelease and Beath, 1949). The first group includes the Beath indicator plants which comprise about twenty-four species and varieties of *Astragalus* (vetch, with pea-like flowers), and all species of *Xylorrhiza* (woody aster), *Oonopsis* (golden weed), and *Stanleya* (princes plume) so far examined. The second group includes a great number of plants which survive without selenium, but selenium generally tends to stimulate their growth. It is obvious, therefore, that a knowledge of these plants would be most useful for the livestock people, in planning a more intelligent grazing operation on ranges containing selenium.

Literature Review

The literature reviewed on selenium and other trace elements does not reveal any detailed studies on wool, but does give information on

[1] Ph.D. thesis in Wool Department. The writers wish to acknowledge help and suggestions by O. A. Beath of the Research Chemistry Department of the University of Wyoming.

the effects of these trace elements on hair, skin, and the general health of domestic animals.

Probably one of the first and most discerning accounts of the then unknown selenium poisoning was written by T. C. Madison (1860), the surgeon at Fort Randall in Nebraska Territory (now Gregory County, South Dakota). This account deals with a fatal disease which appeared in the dragoon horses, in which the feet were affected and the hoof sloughed off at the hair line. It is interesting to note that from about this time until 1934, selenium poisoning became known as "alkali disease" among the ranchers and farmers of this area as it was felt that the disease was caused by alkali in the water and soil. The literature does not indicate any concentrated effort to ascertain the true cause of alkali disease until the early years of this century when the problem was attacked. Beath and his co-workers (1917, 1921, 1932, 1934a, 1934b, 1935, 1936, 1937a, 1937b, 1937c, 1939a, 1939b, 1940, 1941, 1943 and 1947) conducted a most extensive program which included a study of plants, animals, and geological structures of Wyoming and other states, and listed the Beath indicator plants which grow on seleniferous soils.

A large amount of work has been done on selenium fed to laboratory rats which pointed out the lethal nature of the feeds grown on certain types of soils in South Dakota where livestock had shown signs of the so-called alkali disease. Franke (1934) demonstrated that selenium feeding caused anaemia and death in rats, and that protein fractions of wheat and corn grown in certain sections of South Dakota also caused severe symptoms and death. Hurd-Karrer and Kennedy (1936) concluded that wheat, comprising 70 percent of the diet, grown on soil containing 2 ppm of selenium caused retarded growth and liver injury, and wheat grown on similar soil with flowers of sulfur or gypsum added, did not show a toxic effect. Beath and others (1939a) demonstrated that many malformed lambs were the progeny of ewes grazed on seleniferous ranges. The joints and extremities were thickened and nodular in appearance. Most of the lambs died soon after birth, and those that lived had deformed legs and thickened joints with impaired locomotion. The various organs showed pathological changes similar to those found in the "blind stagger" type of poisoning.

Miller and Williams (1940) found that a ration containing 17.5 to 115 ppm of selenium was refused by horses and mules. After two months, selenium was given as a drench. Latterly the selenium caused a loosening of the hair and a softening and scaling of the horny parts of the hoof. Lesions found upon autopsy resembled those described in field cases. Rosenfeld and Beath (1946) pointed out that selenium

poisoning in sheep caused a gradual decrease in Vitamin A and total protein content of the blood. A more rapid decrease in the ascorbic acid, and an increase in nonprotein nitrogen was noted in all sheep dying from selenium poisoning. The heart and liver were affected by selenium feeding while the kidneys, lungs, and spleens showed no changes in total protein, sulfur and nitrogen content. The blood gradually became normal after selenium feeding was discontinued. Rosenfeld and Beath (1947) found that about 75 percent of the malformed lambs died at birth and that 10 percent died when 3–5 months of age. The lambs that lived showed deformities of the eyes and extremities, and hypoplasia of the reproductive organs.

The importance of the so-called trace elements such as copper, cobalt, molybdenum, and selenium on sheep nutrition has been pointed out by various research workers, and they have found these elements to be interrelated in their physiology. Wilson (1951) reports experiments in California indicating that an excess of molybdenum in the sheep's diet will restrict the animal's ability to use any normal amount of copper present and thus produce the effect of a copper shortage. A sheep with a naturally black fleece will change to grey or near-white if copper is absent from the ration, or if molybdenum is present in too great an amount.

The Problem and Experimental Plan

This study was set up to investigate the effect of selenized diets on some of the chemical and physical properties of wool fibers. Thirty ewes of various ages were selected from the flocks of three cooperating sheepmen in the Medicine Bow and Rock River areas where the selenium contents of the soils were known to vary from low to high. During November and December, 1950, blood samples and wool samples were taken from these ewes when the selenium content of the blood and wool was thought to be greatest. They were given individual ear tags and left in the band for breeding. After the breeding season, they were brought to Laramie in January, 1951, and placed in a range band at the Flag Ranch where they were lambed and shorn before being returned to their respective owners. The Flag Ranch range is presumably free of selenium, and the reaction of the ewes to a selenium-free area was the basis of control of this experiment.

Wool samples taken at the beginning of the test indicated the amount of selenium in the wool of ewes grazing on a selenium range, while samples taken at the end of the test indicated the amount of selenium

in the wool of ewes grazing on selenium free range. These samples were shorter than the full length fleece which was divided into selenized and selenium-free portions according to the amount of time the ewes spent on each type of range. Thus the short-growth samples were a check on the lower and upper zones of the full-growth samples.

The Flag Ranch area has no high-protein forages, consequently natural feeds were supplemented with 40 percent cottonseed cake. The ewes of this flock were started with ⅛ lb. of cake per head per day and were gradually increased to ⅓ lb. of cake per head per day during the winter months. During the lambing time the ewes received 1½ lb. of cake per head per day. These ewes were shorn during the middle of June, but due to a misunderstanding, no identification was made of each fleece giving the owner and ear tag number of the sheep. One ewe was lost and one ewe died, so only 28 fleeces were available. All ewes had a lamb at the time of shearing. During the year preceding the experiment, the flocks of the three ranchers were managed similarly. The grazing ranges of the three operators seemed to be very similar in quantity and quality of grass. In this test all the sheep seemed emaciated and listless when they arrived at the Flag Ranch. At shearing time, all sheep seemed to be very alert and in excellent condition.

Samples were taken from the side area of each fleece, making up a sample of around 60 gm. in weight by selecting it at random one lock at a time, from the right side of the fleece which had been laid out flat on an Australian type skirting table. Each lock in the sample was scoured separately to maintain its original shape with the base and tip intact. The samples were scoured in the standard scouring solution used at the Wyoming Wool Laboratory shown in table 1.

The temperature of the scouring solutions was held, as nearly as possible, at 130° F. After they were scoured, the samples were placed in the humidity room which was set at 65 percent relative humidity and at a temperature of 70° F. They were allowed to come into equilibrium, that is, three consecutive weighings at 20-minute intervals gave the same result (A.S.T.M. 1947). The samples were combed by means of the Suter Stapler (A.S.T.M. 1947) and separated out into the different length classes. The fibers were allowed to come into moisture equilibrium again and were weighed to the nearest .0001 gm. Sampling established the following definitions of terms used throughout this study. The selenized portion means the tip end of the staple which will include the first six months' growth while the sheep were on a selenized diet plus one month on a selenium-free diet to allow all or most of the selenium

in the body to be eliminated. Thus the total time of growth considered for the selenized portion would be approximately seven months or 58.3 percent of the fiber length measured from the tip. Consequently, the selenium-free-portion was considered as the base or 41.7 percent of the fiber length measured from the base. Therefore, for chemical analysis, the length groups obtained from the Suter Stapler were measured in mm. and as nearly as possible, cut into lengths prescribed by the above proportions.

TABLE 1. STANDARD SCOURING SOLUTIONS

Bowl Numbers	Strength of Solutions	
	Soap	Soda Ash
	%	%
1	0	1.3
2	0.6	0.9
3	0.3	0.85
4	0	0.40
5	0	0.40
6	0	0.40

The selenium content was found to have a basis for physical and chemical correlations. Furthermore, selenium contents for both the selenized portion of the fiber and the selenium-free portion of the fiber were established in an attempt to indicate the length of time selenium may be active in the body of a sheep. The cut lengths representing the selenized and selenium-free portions were washed in ether to reduce the residual fat content as much as possible. The samples were dried and then carded to achieve homogeneity and were then examined for vegetable matter which was removed if noted. After the samples had reached equilibrium with the standard atmosphere of the humidity room, a 10-gm. sample was taken to be dried and weighed to the nearest 0.01 gm. Drying was accomplished by leaving the samples in an Emerson conditioning oven for not less than 6 hours at 220 degrees F. At this time a 500-mg. sample also was taken for the sulfur analysis. The samples were not analyzed in duplicate as the cost per sample was quite high. In analyzing these samples for selenium content, the method described by Trelease and Beath (1949) for the determination of selenium in plants was followed.

Results

Selenium Content of the Wool Fiber

The wool from 28 ewes was analyzed for selenium content, placing it in two categories, selenized and selenium-free. The selenized portion had a range of 0.0 to 5.0 ppm of selenium with an average of 1.40 ppm. The selenium-free portion, that is the portion grown on a supposedly selenium-free range, contained from 0.0 to 3.7 ppm of selenium with an average of 0.66 ppm. In analyzing by the Paired Method, Snedecor (1940), the results showed a highly significant difference (1 percent level), indicating that the selenium portion (staple tip) contained a greater amount of selenium than the selenium-free portion (staple base). The data were then analyzed for curvilinearity. This analysis gave a curvilinear correlation of .92 and a determination coefficient (R^2) of .85 (highly significant). The t value for curvilinearity was highly significant at the 1 percent level. As the values for the selenized portion of the samples in this test increased, the amount of selenium present in the selenium-free portion increased at an increasing rate. Conversely, the selenium decreases from the selenium-free portion at a rate similar to that from the selenized portion. The sheep had been on a selenium-free feed during the time that thet selenium-free portion of the wool was grown, thus substantiating the conclusion that the selenium had been accumulated in the body. In the 28 sheep in the test, the greater the concentration of selenium in the animal, the slower the animal eliminated the poison. The selenium content of the short-growth fibers checked with that of the selenized and selenium-free zone of the full-growth fibers.

Sulfur Content of Selenized Wools

The sulfur content of the selenized wools was determined to see whether or not selenium was replacing sulfur in the cystine molecule. The selenized portion of the wool fiber was used for sulfur determination. The determination of sulfur was based on the procedure described by Block and Bolling (1948). The sulfur content ranged from .0150 to .01678 gm. with a mean of .01589 gm. The linear correlation (r) between sulfur and selenium was —0.23 (not significant at the 5 percent level). However, the negative correlation is of some interest in that it suggests that the higher the amount of selenium, the lower the concentration of sulfur. As this correlation approaches significance,

more work concerning the relationship of sulfur and selenium may be of some value. Under the conditions of this experiment, the data show no relationship between selenium and sulfur.

Selenium Content and Physical Factors

The physical factors investigated included fiber length, thickness within length groups, tensile strength, and distorted fibers. A multiple regression coefficient was calculated using length of fiber as the dependent variable and selenium and weight of fiber in each length group as independent variables. The data indicated that no significant partial correlation existed between selenium and weight of fiber in each length group (r equals .02), and no significant partial correlation was found between selenium and length groups (r equals .05). However, a highly significant partial correlation existed between length groups and weight of fiber in the length groups (r equals .55), indicating that the longer the fiber, the heavier it will be. The data from these samples indicated that the amount or presence of selenium does not affect the length of fiber or the amount of fiber produced, but the weight of fiber in its respective length groups is governed to a large degree by its length.

The thickness of fibers for each length group and for each fleece were studied to determine whether or not selenium affected the fiber thickness. The short fiber method of determining fiber thickness (A.S.T.M. 1947 Designation D 472–47, T) was followed. One slight deviation was incorporated in that each length group was sampled at the center to determine the thickness of the selenized portion, and again sampled as close to the base as possible to study the selenium-free portion. A review of the literature appeared to indicate that some of the diameter differences of the selenized wool samples was probably due to fetal and nursing factors. The ewes in this test all produced one lamb, and the fiber was measured near the end of pregnancy or at lambing time. Under these relatively similar conditions, all the fibers were probably reduced to about the same degree. Therefore, by using thickness differences the reduction in thickness was held constant in the multiple correlation. The average thickness of the base portions was subtracted from those of the selenized portions to obtain the difference in thickness. The differences ranged from 0.30 to 6.20 microns, with the average difference being 3.01 microns. Length is another factor related to the thickness of wool fibers, and consequently the variables in this analysis were differences of thickness, length of fibers, and selenium. The results of the multiple regression indicated no significant partial correlation between

length and selenium (r equals .20), nor between difference of thickness and selenium (r equals .11), but a highly significant relationship existed between the difference of thickness and length of fiber (r equals .35). The determination coefficient was very low leaving approximately 88 percent of the variation unaccounted for. However, the multiple correlation coefficient was highly significant (R equals .35). In the samples tested, selenium did not significantly affect the length of staple or the thickness differences between groups. There seems to be a high relationship between thickness and length of fiber in these 28 fleeces.

The tensile strength of bundles of fibers for each fleece was determined by the Scott Tester by the procedure outlined in the Standards for textile materials (A.S.T.M. 1950), with modifications so as to obtain portions of the selenized wool. Tensile strength was tested to find whether or not selenium affected the strength of wool fibers. Multiple correlations were calculated for three variables, tensile strength, fiber thickness, and selenium content. The partial correlations between thickness and selenium (r equals .03), thickness and tensile strength (r equals .13), and tensile strength and selenium (r equals .16) were not significant. This was also true for the multiple correlation coefficient (R). In these 28 samples, selenium content or thickness did not affect tensile strength, and thickness was not affected by selenium, which substantiated the information obtained in the thickness studies already reported.

Distortions were noted in the selenized portion of the wool fibers, but were rarely noted in the selenium-free portion of the same samples, while the thickness measurements were being made. The distortion appeared as a distinct enlargement of the fiber, similar to the knot formed when the branch of a tree had been broken off and the scar had healed. At times, the frequency of these distortions was high and at other times, it was quite low. The selenized portion of the staple was sampled and short-sectioned slides were made in the same manner as slides for thickness measurements (A.S.T.M. 1947). One thousand fibers were observed and the distortions on each fiber were counted. The same method was used for the selenium-free portion of the samples. The variables most likely to be affected by this condition (distortions) were tensile strength and selenium. Consequently, a multiple correlation based on three vairables, tensile strength, selenium, and distortions was calculated. The distortions ranged from zero to 204 for each 1,000 fibers observed in the selenized portion of each fleece. The partial correlations between tensile strength and selenium were not significant at the 5 percent level. However, the partial correlation between selenium and dis-

tortions was highly significant at the 1 percent level (r equals .73). Selenium is related to distortions but not to tensile strength. This information further confirmed the results of the strength tests, as tensile strength was again shown to be unaffected by selenium. Furthermore, the distortions did not affect the tensile strength of these samples. However, the information gathered here affords a basis to believe that the more distorted fibers present, the higher the selenium content. In view of the literature reviewed, it was not clear whether the selenium was directly responsible for the distortions, or whether the repellent nature of feeds high in selenium kept the sheep on restricted diets. This may have caused the fiber to become thinner, and when the sheep found relatively palatable and adequate feed, the fiber would again grow normally. However, in view of the fact that the thickness and length of these wools were not significantly affected by selenium, it is assumed that selenium was the causative factor for the distortions found. If a portion of a fiber increased in thickness, it should also increase in rigidity at the point of distortion. Harshness in the wools tested would appear, at least partially, to have been caused by distortions found in the tested samples.

Selenium Content in Blood and Wool

Blood samples (about 25 cc) and wool samples (about 30 gm.) were analyzed from the 30 sheep used in this experiment, during the last week in October and the first ten days of November, to determine whether any correlation existed between wool selenium and blood selenium. The blood was taken from the jugular vein by the University Veterinarian, and at the same time a wool sample was taken from the side of each animal. The samples were analyzed for selenium content at the Research Chemistry Department laboratory by methods outlined by Trelease and Beath (1949). Statistical analysis of the data indicated no significant differences between the levels of blood selenium and wool selenium. Neither a significant linear correlation (r equals .03) nor a significant curvilinear correlation (r equals .12) was found to exist. The averages of the selenium content of the blood and wool gave some interesting results. The sheep from cooperator one, which were grazed on the low selenium area, had a mean blood selenium content of 0.43 ppm, and a mean wool selenium content of 1.51 ppm. The animals from cooperator two, which were on the medium selenium area, had a mean blood selenium content of 1.16 ppm, and a mean wool selenium content of 2.26 ppm. The sheep of cooperator three, which were on the highest

selenium area, had a mean blood selenium content of 1.98 ppm, and a mean wool selenium content of 1.44 ppm. The blood selenium increased as the concentration of selenium in grazing areas increased, but this was not true of the wool selenium which was about the same for the three areas. The data in this analysis do not seem adequate as the blood selenium represented the amount of selenium in the blood at a given moment, and the wool selenium represented an accumulation of selenium over a five months period. Furthermore, the blood and wool samples were taken at a time when the selenium was thought to be most concentrated in the forage. This is significant when it is noted that the wool samples were cut from the sheep with a pair of hand shears which left about ¼ inch of stubble. This stubble would account for about one month of growth, thus giving the blood selenium virtually one month's advantage over wool selenium in forage selenium concentration. This may, in part, explain why no correlation existed between blood and wool selenium contents, and yet a highly significant curvilinear correlation existed between the selenium contents of the selenized portion and the "selenium-free" portion of the wool fiber taken from the same animals. To determine if the blood picture had changed, blood samples were again taken on May 11, 1951, after the sheep had been on a selenium-free area for four-months. Ten sheep were selected, three from Cooperator one, three from Cooperator two, and four from Cooperator three. Of the ten sheep analyzed, results from six sheep were obtained. The blood from these six sheep showed a range of 0.0 ppm to only a trace of selenium, indicating that these six sheep had practically eliminated the selenium in about four months of grazing on range free from selenium.

Summary

Twenty-eight range sheep owned by three sheepmen in the Medicine Bow and Rock River areas of Southern Wyoming were studied to determine whether selenium occurred in the wool fiber, and whether the harshness of these wools could be caused by this poison. The wool was from sheep grazed on known selenium areas. Blood and wool samples were taken in October and November and analyzed for selenium. The results indicated that no significant difference existed between blood selenium and wool selenium. Furthermore, no significant linear or curvilinear correlations could be found with blood selenium as the independent variable and wool selenium as the dependent variable. In this preliminary study, the range sheep in the test were left on the range

throughout the test and complete control of the animals was impossible. The sheep were placed on a selenium-free range during January and remained there until after lambing and shearing in May when they were returned to their home range which was known to carry selenium in the soil and herbage. Prior to shearing, ten sheep were selected at random, and blood samples were taken to determine the selenium content after grazing on a selenium-free range and receiving a supplement consisting of cottonseed cake with a 40 percent protein content. There was a large decrease in blood selenium in these sheep compared to the first blood selenium samples taken in October. A chemical analysis was made which indicated that the more selenium in the body of the animal the slower the selenium left the body. A physical analysis of the wool indicated that selenium did not significantly affect the length of fiber, fiber thickness or fiber strength. The latter factor would indicate that the so-called harshness of wool is not directly associated with selenized wool if fiber strength is taken as the measure of harshness. Selenium content of the wool did have a highly significant correlation with the number of distorted fibers in the fleece.

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THE RESPONSE OF COBALT-DEFICIENT LAMBS TO ORALLY ADMINISTERED VITAMIN B_{12}

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FOLLOWING the discovery that vitamin B_{12} contained cobalt by Rickes *et al.* (1948) and Smith (1948) several investigators proposed that cobalt was required by the rumen bacteria to synthesize vitamin B_{12}. Injection of small amounts of available crystalline vitamin B_{12} failed to alleviate cobalt deficiency as reported by Becker *et al.* (1949), Hale *et al.* (1950) and Marston and Lee (1949). However, as larger quantities of vitamin B_{12} became available Smith *et al.* (1951) demonstrated that the injection of crystalline vitamin B_{12} would cure cobalt-deficiency symptoms in sheep. This was later confirmed by Anderson and Andrews (1952), Hoekstra *et al.* (1952), Marston (1952) and Marston and Smith (1952).

In 1950 Hale *et al.* reported that the oral administration of 200 micrograms of vitamin B_{12} daily for 4 weeks to 1 cobalt-deficient sheep failed to produce a satisfactory response.

The study reported in this paper was undertaken to test the therapeutic value of vitamin B_{12} for cobalt deficient sheep when administered orally in relatively large doses.

Experimental Procedure

Fifteen western yearling wether lambs averaging 80 lb. in weight were group-fed a cobalt-low ration of shelled yellow corn, powdered skimmilk, and a grass hay containing 0.05 ppm of cobalt. On this ration the lambs gained as much as 0.3 lb. per day and reached an average weight of 115 lb. before deficiency symptoms became evident, a period of 5 months.

At this time the lambs were moved to individual pens to permit the accurate recording of individual feed intake. Plain salt blocks were always available and the lambs were offered water once daily. Body weight changes, hemoglobin blood levels, and feed intake were used as measures of the deficiency state.

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Since the sheep did not develop cobalt deficiency at a uniform rate it was not possible to start treatment of all the sheep at one time. However, all were in about the same deficiency state when they were put on a selected treatment. Following treatment, the lambs were kept on the deficient ration until they again exhibited the symptoms of cobalt deficiency. At this time they were given a second treatment which was in no case the same treatment they had previously been given.

The treatments consisted of (1) 500 mcg. of crystalline vitamin B_{12} daily given orally, (2) vitamin B_{12} feeding supplement (Merck) orally which furnished 100 mcg. vitamin B_{12} daily, (3) 100 mcg. of crystalline vitamin B_{12} daily given orally, and (4) negative controls which at a later time served as positive controls, when they were injected with a total of 500 mcg. of vitamin B_{12}.

The length of treatment was not rigidly set prior to treatment. It was planned to continue the treatment until a response was obtained or until it was obvious the lambs would not respond as shown by weight changes, hemoglobin levels, and feed intake.

The lambs which received 500 mcg. of crystalline vitamin B_{12} were dosed twice weekly by pipette for a period of 5 weeks. The lambs fed the feeding supplement were given the supplement by capsule daily for a period of 8 weeks. The lambs fed 100 mcg. of vitamin B_{12} were dosed twice weekly by pipette for a period of 8 weeks. The lambs injected with vitamin B_{12} were treated twice weekly at a rate to furnish 500 mcg. of vitamin B_{12} over a 2-week period.

Results

In all cases body weight and appetite changes were used as primary measures of response. Hemoglobin changes were observed but previous experiments here at Cornell have shown these changes to be erratic and slow following cobalt or vitamin B_{12} therapy.

Table 1 summarizes the average weekly weight changes of the cobalt-deficient lambs following treatment. The weights of the positive control lambs injected with vitamin B_{12} are shown for 4 weeks prior to treatment during which period they lost weight.

The lambs that received 500 mcg. of vitamin B_{12} orally each day exhibited a slightly better weight response than did the positive control lambs, however, both groups reached their maximum weight 3 weeks after the treatment ended. The lambs fed the 100 mcg. of vitamin B_{12} via the feeding supplement showed a loss of weight for the first 3 weeks comparable to the weight loss of the lambs maintained on the cobalt-

TABLE 1. WEIGHT RESPONSES OF TREATED COBALT-DEFICIENT LAMBS

Week	B_{12} Injection (5) (500 mcg./2 wk.)	B_{12} Orally (5) (500 mcg./da.)	B_{12} Orally (3) (100 mcg./da.)	B_{12} Feeding Supplement (3)
0	78±3.2[1]	73±2.5[1]	73±4.4[1]	78±4.7[1]
1	76±3.6	74±2.0	72±4.7	76±5.8
2	76±3.6	74±2.9	70±4.4	76±7.2
3	74±3.4	75±1.9	69±4.2	74±7.0
4	70±3.1[2]	78±1.5	68±4.4	76±6.5
5	75±3.2	81±0.7[3]	67±3.2	78±7.2
6	76±3.2[3]	84±2.2	67±3.9	80±6.8
7	77±3.0	83±2.1	65±5.9	81±6.2
8	79±2.2	85±1.6	66±6.6[3]	83±7.1[3]
9	80±3.9	85±2.6	63±4.9	87±5.0
10	80±5.1	84±2.6	62±5.5	90±5.4

[1] Mean ± standard error.
[2] Started B_{12} injection.
[3] End of treatment.
( ) No. of animals.

deficient ration. However, after this they gained rapidly. The lambs fed crystalline vitamin B_{12} equivalent to that furnished by the feeding supplement continued to lose weight during the 10-week period.

Table 2 presents the average daily concentrate intake of the cobalt-deficient lambs following treatment. All treatment groups showed a

TABLE 2. DAILY CONCENTRATE INTAKE OF TREATED COBALT-DEFICIENT LAMBS

Week	B_{12} Injection (5) (500 mcg./2 wk.)	B_{12} Orally (5) (500 mcg./da.)	B_{12} Orally (3) (100 mcg./da.)	B_{12} Feeding Supplement (3)
	gm.	gm.	gm.	gm.
0	131±37[1]	57±14[1]	39±19[1]	75±20[1]
1	129±46	97±16	51±21	146±25
2	88±41	140±33	46±18	102±38
3	93±42	214±54	32±15	184±67
4	55±12[2]	263±64	26±11	198±71
5	124±15	383±49[3]	32±14	295±59
6	134±38[3]	384±66	49±16	331±99
7	229±40	411±59	55±23	400±64
8	251±67	362±61	27±15[3]	441±39[3]
9	250±78	314±82	19± 7	468±21
10	225±81	281±80	15±..	500± 8

[1] Mean ± standard error.
[2] Started B_{12} injection.
[3] End of treatment.
( ) No. of animals.

positive response except the lambs fed 100 mcg. of crystalline vitamin B_{12} per day. The lambs fed 500 mcg. vitamin B_{12} and those injected with vitamin B_{12} both exhibited maximum daily concentrate intakes the second week after the treatment was discontinued. However, the administration of 500 mcg. of vitamin B_{12} orally stimulated a higher concentrate-intake than did the injection of vitamin B_{12}. The lambs fed the B_{12} feeding supplement were slow to respond.

The hemoglobin responses are presented in table 3. These responses as previously observed, were very variable and delayed. The groups

TABLE 3. HEMOGLOBIN LEVELS OF TREATED COBALT-DEFICIENT LAMBS

Week	B_{12} Injection (5) (500 mcg./2 wk.)	B_{12} Orally (5) (500 mcg./da.)	B_{12} Orally (3) (100 mcg./da.)	B_{12} Feeding Supplement (3)
		gm./100 ml. blood		
0	9.12±0.56[1]	8.10±0.56[1]	9.91±1.58[1]	7.89±0.74[1]
2	8.99±0.74	7.76±0.80	8.55±0.92	7.58±0.32
4	9.65±0.87[2]	8.37±0.28	8.56±1.47	7.00±0.60
6	8.16±0.63	8.91±0.59[3]	7.52±1.45	8.14±0.33
8	8.79±0.53[3]	8.99±0.43	8.28±1.52[3]	9.20±0.59[3]
10	8.86±0.63	10.62±0.30	6.32±....	8.64±0.54

[1] Mean ± standard error.
[2] Started B_{12} injection.
[3] End of treatment.
( ) No. of animals.

that received 500 mcg. of vitamin B_{12} orally and those that received the feeding supplement showed a small but definite response. Those fed 100 mcg. of crystalline vitamin B_{12} exhibited a decrease in hemoglobin level. The lambs that received the vitamin B_{12} injections also showed a decrease in hemoglobin levels. However, the hemoglobin level the twelfth week, which is not shown on the table, averaged 10.02 gm. per 100 ml. of blood, a level somewhat higher than at the start of the treatment.

A summary of weight, appetite, and hemoglobin responses by treatment groups is shown in table 4. The lambs fed the vitamin B_{12} feeding supplement made the best gains and showed the best appetite response. The lambs fed 500 mcg. of vitamin B_{12} made greater gains and showed a greater appetite response than did the positive controls. In all categories, the group which received 100 mcg. of vitamin B_{12} orally each day showed a negative response.

Analyses of the weight changes, concentrate-intake changes, roughage-intake changes and hemoglobin changes using $t = \frac{d}{\sqrt{\frac{1}{n_1} + \frac{1}{n_2}(E)}}$ where E is calculated from the analysis of variance, showed that all measurements for the B_{12} injection, 500 mcg. B_{12} orally and B_{12} feeding supplement groups, except hemoglobin changes for the B_{12} injection group, were significantly greater than for the 100 mcg. B_{12} orally group.

TABLE 4. SUMMARY OF WEIGHT, APPETITE, AND HEMOGLOBIN RESPONSES OF TREATED COBALT-DEFICIENT SHEEP

Comparison	B_{12} Injection (500 mcg./2 wk.)	B_{12} Orally (500 mcg./da.)	B_{12} Orally (100 mcg./da.)	B_{12} Feeding Supplement
No. of animals	5	5	3	3
Length of treatment (weeks)	2	5	8	8
Total B_{12} administered	500 mcg.	17.5 mg.	5.6 mg.	5.6 mg.
Weight changes (av.) (lb.)[1]	10**	12**	−11	16**
Concentrate-intake changes (gm./da.)[1]	196*	353**	−40	425**
Roughage-intake changes (gm./da.)[1]	230**	201**	−155	294**
Hemoglobin changes (gm./100 mg.)[1]	−1.49	2.86**	−3.59	2.20**

[1] Difference between basal level and the maximum or minimum level attained.
* Significant at the 5% level.
** Significant at the 1% level.

The results of this study show that vitamin B_{12} can alleviate cobalt deficiency symptoms when given orally at a rate of 500 mcg. daily for a period of 5 weeks. This can be safely considered a response to vitamin B_{12} *per se* and not to the cobalt contained in the B_{12} molecule. Using a cobalt content of 4.5 percent for vitamin B_{12} as reported by Wolf *et al.* (1950) the daily vitamin B_{12} dose furnished 0.0225 mg. cobalt. The maximum feed offered to any animal furnished an additional 0.0303 mg. of cobalt making a total maximum daily ingestion of 0.0528 mg. cobalt which is less than experiments by Filmer and Underwood (1937), and Marston (1952) have shown to be required by sheep.

The feeding of 500 mcg. of vitamin B_{12} daily for 5 weeks to cobalt-deficient lambs produced as favorable a response in appetite, body weight, and hemoglobin level as the injection of 500 mcg. of vitamin B_{12} over a 2-week period. Assuming these responses to be approximately equal at least 3 percent or more of the orally administered vitamin must have been absorbed from the gastro-intestinal tract.

The positive response of cobalt-deficient lambs to the vitamin B_{12} feeding supplement needs comment. Since comparable cobalt-deficient

lambs fed the same amount of crystalline vitamin B_{12}, i.e. 100 mcg. per day, did not respond, the response to the feeding supplement was due to something other than vitamin B_{12} itself. Subsequent studies revealed that this feeding supplement was rich in cobalt, enough to furnish each lamb about 0.5 mg. per day. Since this amount of cobalt is approximately five times the daily requirement of lambs, the positive response is easily explained as a response to cobalt *per se*.

Summary

The feeding of 500 mcg. of crystalline vitamin B_{12} daily for 5 weeks to cobalt-deficient sheep produced as favorable a response in appetite, body weight, and hemoglobin levels as the injection of 500 mcg. of vitamin B_{12} over a 2-week period. Assuming these responses to be approximately equal, at least 3 percent of the orally administered vitamin must have been absorbed from the gastro-intestinal tract.

The oral administration of a feeding supplement that contributed 100 mcg. of vitamin B_{12} daily was also found to be effective in alleviating cobalt deficiency. However, cobalt analysis of the feeding supplement showed enough cobalt to give a response to cobalt *per se*.

The feeding of 100 mcg. of crystalline vitamin B_{12} daily for 8 weeks failed to elicit a response. The results of this experiment demonstrate that cobalt-deficient lambs will recover when fed vitamin B_{12}, but the effective dose is approximately 35 times as much as the parenteral dose.

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A STUDY OF THE PROTEIN REQUIREMENTS OF FATTENING FEEDER LAMBS [1]

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NUMEROUS experiments have shown that a ration of shelled corn and good alfalfa hay will produce satisfactory gains. But often it is necessary to feed a roughage lower in protein than alfalfa hay. Corn or sorghum silage can be used satisfactorily to replace a part, or all, of the dry roughage, if properly supplemented. When corn or sorghum silage is used in this way, the problem of optimum protein in the ration becomes of economic importance.

When corn silage composed the greater part of the roughage, rations with about 10.3 percent total protein, air-dry basis, have been reported to produce satisfactory gains and finish with feeder lambs. Rations of higher protein percentage did not increase the rate of gain signficantly and tended to produce slightly less finish (Willman *et al.*, 1946; Briggs, 1946; and Rousek, 1943). Later work by Burkitt (1948) with individual feeding trials and Embry (1948, 1950) with feed-lot trials showed that lambs fed rations having about 11.0 percent protein made a more rapid rate of gain than those fed rations containing less protein. However, these workers found some increase in the rate of gain when the percentage of protein in the ration was increased to about 12.0 percent.

The experiments reported here were designed, primarily, to compare the feed-lot performance of lambs fed rations with about 10.0, 11.0, and 11.8 percent total protein.

Procedure

Three experiments were conducted under feed-lot conditions to obtain information on the protein requirement of growing and fattening lambs. In each experiment, a 3 $\times$ 3 factorial arrangement of nine lots of eleven lambs each were used. This design made it possible to determine the relative efficiency of various rations with three levels of total

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protein and also, to compare the feed-lot performance of lambs getting corn silage as the only roughage with similar lambs fed 0.75 lb. and 0.50 lb. of alfalfa hay with a full feed of corn silage.

In these experiments, a total of 297 western lambs was used. White-faced lambs showing a predominance of Rambouillet and Corriedale breeding were used in two of the experiments and black-faced lambs, chiefly of Hampshire breeding, were used in the other experiment. The lambs were weighed individually every two weeks during the experiments. Only one weighing was made at the start and conclusion of each experiment. The lambs were randomly alloted to the lots after grouping according to weight and sex. Prior to the start of each experiment, the lambs were treated for internal and external parasites and vaccinated against "overeating disease."

The lambs in all lots were full fed yellow shelled corn and corn silage, and given free access to salt with trace amounts of cobalt and copper added. The level of total protein was controlled by adjusting the amount of linseed meal added to the ration. A Ca-P ratio of approximately 2 to 1 was maintained by adding ground limestone to the ration when necessary. The limestone was mixed with the linseed meal in the desired proportion for each lot. All feeds were fed twice daily, except when small amounts of linseed meal were fed. A constant amount of hay was fed to each lot according to the experimental plan. The lambs were charged with all of the feed offered. At the conclusion of each experiment, the lambs were graded for finish and assigned the following numerical values: prime—4, choice—3, good—2, and utility—1.

The measurements of performance were the rate of gain, feed efficiency and degree of finish.

Results and Discussion

An analysis of variance of the data according to the method outlined by Snedecor (1948) showed that the interaction of the protein and hay levels was too small to be significant. Therefore, the response to the levels of total protein in the ration is probably independent of the amount of alfalfa hay fed.

Protein Levels

A summary of the three experiments is presented in table 1. The rate of gain made by lambs fed 10.0 or 11.0 percent protein in the ration was about the same, 0.351 and 0.353 lb., respectively. Lambs fed the 11.8 percent protein ration made the most rapid rate of gain regardless

TABLE 1. RESULTS OF FEEDING RATIONS WITH THREE LEVELS OF PROTEIN AND VARIOUS PROPORTIONS OF ALFALFA HAY AND CORN SILAGE

(Summary of Three Experiments, 1950–53)

Per cent protein in air dry feed	10.0			11.0			11.8		
Hay level, lb. per day	0.75	0.50	None	0.75	0.50	none	0.75	0.50	none
Lot No.	I	V	VIII	II	VI	IX	III	VII	X
Av. protein fed, %	10.1	10.0	10.0	11.0	11.0	11.0	11.9	11.8	11.8
No. of lambs	33	33	33	33	33	33	33	33	33
Death loss	0	0	4	0	2	0	0	1	0
Av. initial wt., lb.	58.3	59.5	58.2	58.8	58.1	59.2	58.0	58.4	59.0
Av. final wt., lb.	96.8	99.5	100.1	99.0	100.4	99.0	100.0	102.1	100.7
Av. daily gain, lb.	0.34	0.35	0.36	0.35	0.36	0.35	0.37	0.38	0.37
Av. daily ration, lb.									
Shelled corn	1.21	1.16	1.04	1.13	1.07	0.96	1.03	0.98	0.89
Linseed meal[1]	0.02	0.07	0.16	0.10	0.15	0.25	0.20	0.24	0.33
Alfalfa hay	0.75	0.50	....	0.75	0.50	....	0.75	0.50	....
Corn silage	1.70	2.15	3.24	1.92	2.33	3.31	2.01	2.53	3.62
Ground limestone[2]	....	0.005	0.019	0.002	0.007	0.021	0.004	0.009	0.022
Salt, free choice	0.026	0.023	0.028	0.026	0.024	0.024	0.027	0.025	0.023
Amount of feed per cwt. gain, lb.									
Shelled corn	356.3	319.5	292.2	320.7	300.7	276.2	279.4	261.0	243.9
Linseed meal[1]	4.9	18.1	45.7	29.6	41.5	71.5	54.6	64.3	89.7
Alfalfa hay	221.8	143.1	....	213.7	140.3	....	204.1	133.1	....
Corn silage	499.1	610.6	904.7	545.9	654.3	947.2	541.2	671.0	996.3
Ground limestone[2]	....	1.4	5.3	0.5	2.0	6.0	1.1	2.6	6.2
Salt	7.7	6.6	7.7	7.5	6.6	6.8	7.4	6.8	6.3
Feed costs per cwt. gain, $[3]	17.03	16.33	15.75	17.14	16.52	16.50	16.67	16.15	16.38
Grade on foot[4]	2.9	3.1	3.0	3.3	3.2	3.0	2.9	3.0	2.9

[1] Linseed meal was not fed to lot I in one experiment.
[2] Ground limestone was not fed to lot II in one experiment.
[3] Feed prices per ton: ($) Shelled corn, 68.00; linseed meal, 91.00; alfalfa hay, 24.20; corn silage, 7.80; ground limestone, 7.65; salt, 27.20.
[4] Numerical value for grades: Prime—4, choice—3, good—2, utility—1.

of the amount of hay in the ration. These lambs made an average daily gain of 0.371 lb. which was significantly larger ($P<.05$) than that made by lambs fed 10.0 percent protein. The difference in gains made by lambs fed the highest percentage of protein and those fed the medium level approached significance at the 5 percent level (0.371 vs. 0.353 lb.). Although the lambs fed the highest protein ration made the most rapid gains, they were not quite as fat at market weight as the lambs fed less percentage of protein in their rations. Lambs on the medium level of protein graded significantly higher ($P<.05$) on foot than those fed 11.8 percent protein (3.23 vs. 2.94).

The percentage of protein in the ration had little effect on feed efficiency. Lambs fed the highest level required slightly less total air-dry feed per unit of gain than lambs fed the other levels of protein. The total air-dry feed required per 100 lb. of gain and also, feed costs per 100 lb. of gain were slightly the highest for lambs fed the medium level of protein. Corn silage was reduced to a 12 percent moisture basis when calculating the total air-dry feed required per 100 lb. of gain.

Hay Levels

The amount of hay in the ration did not significantly affect the rate of gain or degree of finish. The average daily rate of gain for the lambs fed 0.75 lb. of hay, 0.50 lb. of hay, and corn silage as the only roughage were 0.355, 0.362, and 0.359 lb., respectively. Lambs offered 0.50 lb. of alfalfa hay and a full feed of corn silage had slightly more finish than the other lambs.

The feed efficiency was increased materially as the amount of hay in the ration was decreased. The total amount of air-dry feed required per 100 lb. of gain and the feed cost per 100 lb. of gain decreased as the amount of alfalfa hay in the ration was decreased, being lowest for the lambs fed corn silage as the only roughage. The feed efficiency was increased significantly ($P<.05$) with each decrease in the amount of hay offered.

In comparing the roughage required per unit of gain by lambs fed 0.75 and 0.50 lb. of hay plus a full feed of corn silage, it was found that 100 lb. of silage saved 64 lb. of alfalfa hay in making an equal amount of gain. In a similar comparison with lambs fed 0.50 lb. of hay and a full feed of silage and lambs fed silage as the only roughage, corn silage was worth only 46 percent as much as hay in terms of hay saved per 100 lb. of gain. This lower value for corn silage when fed as the only roughage is in agreement with work by Willman and Morrison

(1938) and Embry (1950). A better performance may be expected when some hay is included in the ration with a full feed of corn silage.

Summary

A total of 297 western feeder lambs have been used in three experiments. A 3 × 3 factorial arrangement of 9 lots of 11 lambs each was used to determine the value of rations with three levels of total protein, air-dry basis, and various proportions of alfalfa hay and corn silage. Performance was measured in terms of rate of gain, feed required per 100 lb. of gain, and degree of finish.

The lambs fed rations with about 11.8 percent total protein made more rapid gains but were not as fat as those fed rations containing 10.0 and 11.0 percent protein. There was little difference in feed efficiency of lambs fed rations with the various percentages of protein.

The feeding of different amounts of alfalfa hay with a full feed of corn silage did not significantly affect the rate of gain or degree of finish. Lambs fed corn silage as the only roughage made more efficient and economical gains than those fed 0.75 or 0.50 lb. of hay and a full feed of silage. Corn silage has a high value when full fed with 0.50 lb. of alfalfa hay per lamb daily. A better feed-lot performance is obtained when a small amount of hay is fed than when corn silage is fed as the only roughage.

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SOME EFFECTS OF ESTROGEN INJECTIONS ON THE ESTRUAL CYCLE OF GILTS [1]

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THE inhibitory effect of estrogen on the ovaries resulting from decreased pituitary activity was reported by Leonard *et al.* (1931). It was later demonstrated that the effect of estrogen on the pituitary was an inhibition of the secretion of F.S.H. (Frank *et al.*, 1935) and an increased secretion of L. H. (Hohlweg and Junkman, 1932).

The following experiment is a preliminary attempt to characterize the effects of estrogen on the estrual cycle of gilts; also to determine a dosage and time of the cycle for treatment which eventually might be used to modify the estrual cycle so as to facilitate controlled breeding.

Experimental

The 12 gilts assigned to this experiment were of mixed breeding (predominantly Chester White). They were purchased from various farmers at a local market.

The gilts were injected with 3 mg. of diethylstilbestrol in corn oil in the ham region, a dosage which was not expected to produce estrus uniformly. A 3 inch 16 gauge needle was used to insure that the site of deposition was intramuscular rather than into the subcutaneous fatty tissue.

The experiment was so designed that each gilt was to receive during each of three estrual cycles (treatment cycles) an injection of diethylstilbestrol on either the 6th, 11th, or 16th day. (The day heat was detected was considered the first day.) It was intended that four gilts should be started on one injection on the 6th day, followed by one on the 11th day of another cycle and one on the 16th day of another (6-11-16), four others to be started on an 11th day followed by a 16th and a 6th (11-16-6), and the other four on a 16th day followed by a 6th and an 11th (16-6-11). A recovery cycle was to intervene between the 1st and 2nd treatment cycles and also between the 2nd and 3rd.

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The plan was not realized fully. One of the gilts assigned to the 6-11-16 sequence proved to be pregnant; another in the same group was accidentally injected on the day she was expected in heat (but not in) rather than on the 6th day and she did not return to heat before being slaughtered 60 days later. Also one gilt in the 11-16-6 sequence when injected on the 16th day had not returned in heat when killed on the 47th day, hence did not furnish information on a 6th day injection.

Heat checks with a boar were made once a day throughout the experiment. The indication of heat was the willingness of the gilt to allow the boar to mount.

Results and Discussion

The response in length of estrual cycle in the gilts was different at each period of injection (table 1). When injections were made on the 6th day of the cycle there appeared to be no effect insofar as change in length of estrual cycle was concerned. The injection cycles averaged 19.78 days in length while the recovery cycles averaged 19.00 days, the difference not being significant statistically. In those injection cycles in which treatment was given on the 11th day, however, there was a highly significant lengthening, the average being 25.60 days compared to 19.67 days of the recovery cycles. There was a significant effect on the estrual cycle in those in which the injection was made on the 16th day, the average length of these cycles being 17.75 days as compared to 19.86 for the recovery cycles. (This is excepting 2 animals which did not return in heat, see table 1.) The recovery cycles from the various treatment cycles do not differ significantly from each other; however, the difference between the three different treatment cycles is highly significant.

The response of gilts to injections of 3 mg. of diethylstilbestrol appears to depend upon the stage of the estrual cycle at which the injection is given. Injections on the 6th day did not appear to disturb the estrual cycle; injections on the 11th day extended the cycle by approximately 6 days; injections on the 16th day either shortened the cycle by approximately 2 days or prolonged it markedly. The most consistent action, however, was that observed from the 11th-day injection and it was given further study.

The level of fertility that might be exhibited following the long treatment cycles was determined. Three animals injected on the 11th day at their 3rd treatment period and which came into heat on the 24th, 25th and 28th days, respectively, were bred at that time. They were killed

three days later, their reproductive tracts removed and the oviduct and upper one third of the uterus flushed with physiological saline. Fourteen, eleven, and twelve ova were recovered from the three gilts, respectively. All the ova were fertilized. It would thus appear that lengthening of the estrual cycle does not lower the ability of ova matured at the end of long cycles to be fertilized.

TABLE 1. EFFECTS ON LENGTH OF ESTRUAL CYCLE OF INJECTIONS OF DIETHYLSTILBESTROL AT DIFFERENT STAGES OF THE CYCLE IN GILTS

		Day of Cycle When Injected					
		6th		11th		16th	
Cycle	Sequence	No. Cycles	Mean Length	No. Cycles	Mean Length	No. Cycles	Mean Length
Treatment	6-11-16[c]	2	20.00	2	26.00	2	18[a]
Recovery	do.	2	19.50	2	20.00	–	—
Treatment	11-16-6	3	20.00	4	24.75	4	17.70[b]
Recovery	do.	–	——	4	19.50	4	19.50
Treatment	16-6-11	4	19.50	4	26.25	4	17.75
Recovery	do.	4	18.75	–	——	4	19.50
Treatment	All	9	19.78	10	25.60	10	17.75
Recovery	do.	6	19.00	6	19.67	7	19.86

[a] One animal only; the other had not returned in heat when slaughtered on the 26th day.
[b] Three animals only; the other had not returned in heat when slaughtered on the 47th day.
[c] Injection made on 6th day of 1st treatment cycle, 11th day of 2nd and 16th day of third; a recovery cycle followed each of the 1st and 2nd treatment cycles but not the 3rd.

The ovarian condition in animals during the prolonged treatment cycles also was determined in 5 gilts after they had completed the main experiment. They were injected on the 11th day and killed on the 15th day. The findings were very similar in all 5 gilts. They had from 2 to 18 corpora lutea, 10–12 mm. in diameter, which appeared to be normal for that stage in the cycle. All 5 gilts had from one to three luteinized follicles which varied in size from 12 to 35 mm. and in addition, the ovaries of one gilt contained one apparently freshly formed corpus luteum.

The extension of the cycle appears to have been caused by the luteinization of follicles, since this condition was found in all gilts slaughtered following injection at that time. A possible explanation of this luteinization is that estrogen given during midcycle effects the release of a small amount of luteinizing hormone from the anterior pituitary

which results in luteinization of some follicles and perhaps even ovulation of an occasional follicle.

The variability following injection at the 16th day was great; estrus was either hastened or markedly-delayed. Whether ovulation was induced when estrus was hastened has not been established by direct observation of the ovaries, but the length of the following recovery cycle (19.86 days) may be considered as indirect evidence that ovulation occurred at the time of the induced heat rather than at the time it would have occurred normally, an occurrence which would have been expected to lengthen the recovery cycle. The hastening of heat and ovulation when the injection is given as the corpus luteum has begun to wane is perhaps not surprising in view of the hypothesis that estrogen causes the release of L.H. which brings about preovulatory swelling of the follicles and ovulation.

The two gilts which were injected on the 16th day and never returned to heat were killed on the 26th and 47th days, respectively. Normal appearing corpora lutea were present in the ovaries of both, and their endometria were extremely edematous. A third gilt was accidentally injected on the day she was expected in heat. She never returned to heat and was killed 60 days later. Her uterus and ovaries were very similar to the two gilts mentioned above, in that the corpora lutea appeared normal and the endometrium was extremely edematous. Another characteristic exhibited by all three gilts was an extremely small vulva. Its appearance was similar to that of the vulva during the early stages of pregnancy.

The reason for the failure of these gilts to return to heat is obscure. It would be consistent with the explanation for the"estrus-hastened" gilts to suspect that ovulation in these animals was induced early after injection without the expression of estrus. The mechanism for prolonged maintenace of the corpura lutea may also have been set in action. The two 16 day gilts may actually have been nearer to heat than the usual animal at that stage and somewhat comparable to the animal injected on the day she was expected in heat. If so, injection on the 16th day generally would induce ovulation. Heat would occur, however, only when the corpora lutea present were functioning within certain limits; it would not be expressed if the corpora lutea had regressed farther.

It is apparent that further characterization of ovarian changes will necessitate either slaughtering or laparotomizing animals three or four days after injection in order to observe changes in the ovaries. This is particularly true of the 6th and 16th day where as yet no direct observational data on the ovaries are available.

Summary

An experiment has been conducted on 10 gilts which were given intramuscular injections of 3 mg. of diethylstilbestrol in corn oil on either the 6th, 11th, or 16th day of the estrual cycle. Injections on the 11th day were observed to lengthen the estrual cycle significantly, (apparently due to luteinization of follicles), while injections on the 16th day were variable in effect but most frequently caused a significant shortening of the cycle. Injections given on the 6th day of the cycle had no apparent effect. The average lengths of the completed cycles were 19.78, 25.60 and 17.75 days for those in which the injection was made on the 6th, 11th or 16th day, respectively. The corresponding recovery cycles were 19.00, 19.67 and 19.86 days.

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THE EFFECT OF ORALLY ADMINISTERED STILBESTROL AND TESTOSTERONE ON GROWTH AND CARCASS COMPOSITION OF SWINE [1]

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THE growth-stimulatory effect of injected or implanted hormones or hormone-like materials has been known for several years for sheep (Andrews *et al.*, 1949) and cattle (Andrews *et al.*, 1950). More recent research has shown the growth-stimulatory effect of orally administered hormone-like materials for cattle (Burroughs *et al.*, 1954; Perry *et al.*, 1955) and sheep (Hale *et al.*, 1954).

Hormone implantation in swine has had little effect on growth rate or carcass quality (Dinusson *et al.*, 1951; Woehling *et al.*, 1951). However research concerning the effect of orally administered hormones for swine has not been reported.

The experiment reported in this paper was designed to study the effect of orally administered diethylstilbestrol and methyl testosterone on growth rate, feed efficiency and carcass quality of growing and fattening swine.

Experimental Procedure

Sixty weanling Duroc pigs averaging 45 lb. at 9 weeks of age were divided into 6 lots of 10 pigs each on the basis of sex and liveweight. The following treatments were used:

Lot I *Negative Control*
Corn, free-choice, protein supplement (50 soybean oil meal, 40 meat scrap, 10 alfalfa, B-vitamins) free-choice, 40 steamed bonemeal—40 ground limestone—20 salt free-choice.

Lot II *Positive Control*
Same as Lot I except oxytetracycline (terramycin) was incorporated in the free-choice protein supplement at the level of 60 gm. per ton.

Lot III *Stilbestrol Plus Negative Control*
Basal ration indicated in Lot I plus 2.0 mg. stilbestrol per animal daily.

[1] Contribution from the Department of Animal Husbandry, Journal Paper No. 834. Purdue University Agricultural Experiment Station, Lafayette, Indiana.

Lot IV *Stilbestrol Plus Positive Control*
Basal ration indicated in Lot II plus 2.0 mg. stilbestrol per animal daily.

Lot V *Testosterone Plus Negative Control*
Basal ration indicated for Lot I plus 20 mg. testosterone per animal daily.

Lot VI *Testosterone Plus Positive Control*
Basal ration indicated for Lot II plus 20 mg. testosterone per animal daily.

In the indicated hormone treatments, the hormone was dissolved in corn oil, mixed with a small amount of soybean oil meal and finally blended with a 500-lb. mix of protein supplement. The protein supplement was fed on a free choice basis.

The swine were slaughtered at a weight of 220–230 lb. Standard carcass measurements and weights of primal cuts were obtained. One-half of each carcass was boned and skinned and the edible portion was ground and thoroughly mixed. A sample of the ground portion was obtained for analysis of moisture, ether extract and crude protein according to standard analytical procedures.

Samples of ground material were obtained from each animal for estrogen assay as described by Stob *et al.* (1954).

Results and Discussion

Effects of Orally Administered Estrogen and Androgen on Growth Rate

Neither of the orally-administered hormones caused a statistically significant increase in growth rate. (table 1.) Although the feeding of terramycin (Lot II) resulted in significantly increased rate of gain (P less than 0.05) over the negative control (Lot I), the combination of antibiotic and stilbestrol (Lot IV) did not produce a statistically significant increase in gain. Animals receiving the combination of terramycin and testosterone (Lot VI) grew at a significantly slower rate than the animals receiving terramycin but no androgen. The feeding of hormones had no effect on feed consumption or feed efficiency.

Anatomical and Physiological Effects of Estrogens and Androgens

The feeding of stilbestrol produced several marked effects. In the females there was a marked increase in mammary tissue and growth of the teats and an increase in size and congestion of the vulva. In the barrows mammary stimulation was similar to that of the females. These

observations are in agreement with those for stilbestrol-implanted pigs as reported by Dinusson *et al.* (1951) and Woehling *et al.* (1951).

Originally it had been planned to increase the level of stilbestrol in the supplement when the pigs reached 125 lb. liveweight. However, within two weeks after increasing the level of stilbestrol the side effects were so marked that the stilbestrol intake was reduced to the original level.

TABLE 1. THE EFFECT OF ORAL HORMONES ON GROWTH RATE, FEED CONSUMPTION AND FEED EFFICIENCY

	No Antibiotic			Terramycin [1]		
	Control	Stilbestrol	Testosterone	Control	Stilbestrol	Testosterone
Daily Hormone Level	0	2 mg.	20 mg.	0	2 mg.	20 mg.
Initial wt., lb.	45	45	45	46	45	46
Final wt., lb.	223	223	224	226	224	222
Av. daily gain, lb.	1.47	1.52	1.51	1.60[2]	1.56	1.48
			Daily Feed			
Corn, lb.	4.8	4.6	4.9	5.0	5.1	4.8
Suppl., lb.	.45	.60	.62	.55	.64	.59
			Feed per Pound Gain			
Corn, lb.	3.7	3.2	3.3	3.3	3.3	3.4
Suppl., lb.	.34	.42	.43	.42	.42	.41

[1] Terramycin added to free choice protein supplement at a level of 60 grams per ton.
[2] L.S.M.D., 5%, for average daily gain is 0.10 lb.

The testosterone-fed pigs appeared to be somewhat trimmer in the sides and jowls. No objectionable external side effects were observed.

Effect of Orally Administered Hormones on Weight of Primal Cuts.

The feeding of testosterone with or without terramycin resulted in the production of hams that were significantly heavier (P less than 0.05) than those from animals receiving no hormone (table 2) (14.8 and 14.9 vs. 14.0 and 14.1 lb., respectively). The feeding of stilbestrol had no significant effect on the weight of hams produced.

The same trend was true in the case of another lean cut, the picnic. Testosterone-fed pigs, either with or without the antibiotic, produced significantly heavier (P less than 0.05) picnics than those produced by the negative control group (Lot I).

Testosterone or stilbestrol, when fed in combination with terramycin, caused the production of loins (12.0 and 11.8 lb.) which were significantly heavier (P less than 0.05) than those from the control group receiving no hormone or no antibiotic (11.1 lb.).

In following the trend of leaner carcasses from testosterone-fed swine, the weights of the fat cuts were heaviest for the antibiotic-fed pigs receiving no hormone, followed by pigs receiving neither hormone or antibiotic, then by stilbestrol-fed pigs and finally by testosterone-fed pigs. Antibiotic-fed pigs which received no hormone produced fat backs (9.4

TABLE 2. THE EFFECT OF ORAL HORMONES ON DRESSING PERCENT, CARCASS MEASUREMENTS, WEIGHT OF PRIMAL CUTS AND CARCASS CHEMICAL ANALYSIS

	No Antibiotic			Terramycin		
	Control I	Stilbestrol III	Testosterone V	Control II	Stilbestrol IV	Testosterone VI
Pre-kill wt., lb.	220	219	220	223	219	218
Chilled wt., lb.	159	156	155	159	155	156
Dressing percent	72.3	71.2	70.4	71.3	70.8	71.6
Carcass Measurements						
Carcass length, in.	29.6	29.6	32.9	29.7	30.4	30.1
Leg length, in.	20.0	20.4	20.6	20.2	20.6	20.6
Back fat depth, M.M.	52[1]	51[1]	49	53[1]	48	47
Wt. Primal Cuts						
Ham wt., lb.	14.0	14.1	14.9[2]	14.1	14.6	14.8[2]
Loin wt., lb.	11.1	11.2	11.5	11.4	11.8[3]	12.0[3]
Boston butt wt., lb.	5.2	5.1	5.2	5.0	5.2	5.3
Picnic wt., lb.	6.6	6.8	7.0[4]	6.7	6.8	7.0[4]
Belly wt., lb.	13.7[5]	13.0	12.6	13.9[5]	12.8	12.7
Fat back wt., lb.	8.9[6]	8.2	7.9	9.4[6]	8.1	8.1
Jowl wt., lb.	3.3[7]	3.2[7]	3.0	3.2[7]	3.2[7]	2.8
Chemical Analysis Edible Portion						
Moisture, %	35.1	36.7	39.4	35.1	37.5	37.9
Fat, %	55.1[8]	53.7[8]	50.0	55.4[8]	52.2	50.3
Protein, %	8.2	8.5	9.3	8.2	8.8	9.5

[1] L.S.M.D., 5%, for back fat depth is 2mm.
[2] L.S.M.D., 5%, for ham weight is 0.7 lb.
[3] L.S.M.D., 5%, for loin weight is 0.7 lb.
[4] L.S.M.D., 5%, for picnic weight is 0.4 lb.
[5] L.S.M.D., 5%, for belly weight is 0.8 lb.
[6] L.S.M.D., 5%, for fat back weight is 0.8 lb.
[7] L.S.M.D., 5%, for jowl weight is 0.3 lb.
[8] L.S.M.D., 1%, for percent fat is 5.1; at 5%, 3.8.

lb.) which were significantly heavier (P less than 0.05) than those produced in the negative control lot (Lot I) or in any hormone-fed lot. The production of more body fat from the feeding of antibiotics is in agreement with reports by Perry *et al.* (1953) and Vestal (1951). Similarly, both Lots I and II produced bellies and jowls which were significantly heavier than those produced in both of the testosterone-fed groups. Although the differences were not statistically significant, there was a trend toward the production of leaner carcasses in both of the stilbestrol-fed lots.

The Effect of Orally Administered Hormones on Carcass Composition

The feeding of testosterone, with or without terramycin, resulted in the production of carcasses which contained significantly less (P less than 0.05) fat, and a trend toward more protein and more moisture than pigs receiving either stilbestrol or no hormone. (table II.)

Similarly, the feeding of testosterone, with or without terramycin or the feeding of stilbestrol with terramycin, resulted in the deposition of significantly less (P less than 0.05) back fat (47 to 49 mm. vs. 51 to 53 mm.).

TABLE 3. RESULTS OF ASSAY OF MEAT FROM HORMONE-FED SWINE

Lot	Treatment	No. of Mice	Body Wt.	Uterine Wt.	% of Body Weight
			mg.	mg.	
I	Control	8	30	17.6	.058
II	Control plus terramycin	10	30	16.7	.056
III	Control plus stilbestrol	9	29	15.4	.053
IV	Control plus terramycin plus stilbestrol	9	29	17.2	.060

Effect of Orally Administered Hormones on Deposition in the Carcass

The feeding of stilbestrol to swine (table 3) did not result in deposition of estrogens or estrogen-like materials in the edible portion of the carcass as indicated by the mouse uterine weight assay technique as outlined by Stob *et al.* (1954). This is in agreement with the assay of the carcasses of steers which had received oral hormone-like materials as reported by Perry *et al.* (1955). The meat was not assayed for androgen activity.

Conclusions

Methyl testosterone, at a level of 20 mg. per animal daily, or diethylstilbestrol, at a level of 2 mg. per animal daily, was fed both with and without terramycin in a practical ration to growing and fattening pigs in drylot. The oral administration of these hormones did not improve growth rate or feed efficiency.

The feeding of stilbestrol produced clear-cut mammary stimulation in both males and females and enlargement and congestion of the vulva in the female.

The feeding of testosterone appeared to improve the appearance of the sides and jowls and masculine characteristics were observed.

The carcasses from testosterone-fed pigs contained heavier lean cuts (hams, loins, picnics and boston butts) and lighter fat cuts (fat backs, bellies and jowls) than the carcasses from either the controls receiving no antibiotic or the controls which received terramycin. The four lean cuts represented 62.4 percent of the weight of the cuts from the carcasses of testosterone-fed pigs whereas they represented 58.8 percent of the weight of the cuts in the case of the controls. Similarly, the three fat cuts represented 37.6 percent of the cuts for the testosterone-fed and 41.2 percent of the cuts for animals receiving no hormones. Back fat depth measurements and analysis of the edible portion of one-half carcass from each pig followed the trend of primal cut weights. Carcasses from stilbestrol-fed pigs were intermediate in fat content between control animals and testosterone-fed animals.

Chemical analysis of carcasses showed that the pig receiving testosterone had about 5 percent less fat and 5 percent more lean than the controls.

Assay of the carcasses from the stilbestrol-fed animals failed to reveal any estrogenic activity attributable to treatments.

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PROTEIN LEVELS FOR PIGS AS STUDIED BY GROWTH AND SELF-SELECTION

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THE protein needs of growing swine have been determined under a number of different conditions. Methods of feeding have included *ad libitum* feeding in groups, individual full feeding and individual controlled feeding, using rate of gain and feed efficiency as criteria. Other criteria such as nitrogen retention, carcass analysis, biological value and carcass measurements have been used in studies of the protein needs of swine. In previous studies the character or quantity of protein, fat, carbohydrates, minerals, vitamin and antibiotics have varied over a wide range. Although it is a general opinion that it is desirable to determine the requirements under varied conditions, recent results indicate that variation of the factors involved in the method of approach, or in the diet employed, may readily have a bearing on the result. Likewise, the investigator's opinion of the correct interpretation of the result may strongly influence the conclusion.

Using well-fortified, practical diets the experiments reported here were studies of the effect of protein level on growth.[3] The protein levels selected by pigs fed corn and supplement, free choice, were also observed.

Experimental

Growth tests

Growth tests were conducted on both drylot and pasture. In drylot 40 weanling Hampshire and Hampshire × Yorkshire pigs weighing an average of 31 lb. were selected from 7 litters to form 10 outcome groups of 4 pigs each on the basis of litter, weight and general con-

[1] This material is based upon part of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree at the University of Illinois. Present address: Department of Animal Husbandry, The University of Georgia, Athens, Georgia.

[2] The authors wish to acknowledge Central Soya Co., Inc., Decatur, Indiana; A. E. Staley Mfg. Co., Decatur, Illinois; Merck and Co., Rahway, New Jersey, and Lederle Laboratories, Pearl River, New York, for funds or products which made this investigation possible.

[3] Referred to as increase in weight during the growing period.

dition. Nine of the 10 outcome groups were littermates. The pigs were allotted at random from these groups to 4 lots fed 10, 12, 14 or 16 percent protein rations. The outcome groups were allotted at random to 2 replicates, thus providing 8 pens of 5 pigs each, 2 pens for each protein level. Each pen of pigs was self-fed *ad libitum,* using a 3-hole self-feeder. Each pen of pigs occupied a concrete pen, 7 by 14 ft. with a house 6 by 7½ ft. drawn up to it. Water was supplied at least twice daily until the weather permitted installation of automatic waterers.

The pigs were weighed weekly during the study. The data on gains and feed efficiency were collected in two phases. Phase 1 extended from the beginning of the test to the weigh day on which at least one pen first averaged at least 100 lb. Phase 2 extended from the beginning of the test to the weigh day on which at least one pen first averaged 200 lb.

On pasture 40 weanling Hampshire, Yorkshire, and Hampshire × Yorkshire pigs, weighing an average of 29 lb., were selected from 9 litters to form 10 outcome groups of 4 pigs each on the basis of litter, weight and general condition. Each outcome group consisted of littermates. The pigs were allotted at random from these groups to 4 lots fed rations containing 8, 10, 12 or 14 percent protein. The 10 pigs on each treatment were self fed *ad libitum,* using 6-hole self-feeders. Each lot of pigs was located on a one-half acre tract of alfalfa pasture. Water was supplied at least twice daily until the weather permitted installation of automatic waterers.

The pigs were weighed at 14-day intervals during the test. The data on gains and feed efficiency were divided into 2 phases in the manner of the drylot test, and these likewise were called phase 1 and 2.

The ingredients of the rations used on both drylot and pasture are presented in table 1.

Protein Selection Tests

Protein selection tests were conducted on both drylot and pasture. In drylot 18 weanling Hampshire and Hampshire × Yorkshire pigs, weighing an average of 29 lb., were selected from the same 7 litters used in the drylot growth test and paired on the basis of litter, weight and general condition. Six of the 9 pairs were littermates. The pairs were divided at random between two lots offered, free choice, the same yellow corn, either ground or shelled, plus the same protein supplement as that used in the drylot growth test. The supplement included

the same addition of minerals, vitamins and an antibiotic as the 14-percent-protein ration in the drylot growth test. In addition, mineral mix 3 was supplied free choice. Each lot of pigs was started on test in concrete pens 7 by 14 ft. and later moved to pens inside a barn 8 by 10 ft. with concrete areas 8 by 16 ft. outside the barn to allow more room. The pigs were fed and watered, and their liveweight and feed

TABLE 1. COMPOSITION OF RATIONS FED IN DRYLOT AND PASTURE GROWTH TESTS

Ingredients	Drylot	Pasture
	%	%
Ground yellow corn (7.88% protein) and protein supplement [1, 2]	97.4	97.85
Mineral mixture no. 3 [3]	2.0	2.00
Antibiotic and vitamin B_{12} supplement [4]	0.1	0.10
Vitamin mixture [5]	+	0.05
Vitamin A and D oil (3000A, 600D)	0.5	....

[1] The proportions were varied to yield the desired protein levels.

[2] In drylot the supplement consisted of 50% solvent soybean oil meal, 15% dehydrated alfalfa meal, 15% meat and bone scrap (50% protein), 15% menhaden fish meal and 5% condensed menhaden fish solubles. By analysis (N × 6.25) it contained 43.63% protein. On pasture the supplement consisted of 60% soybean oil meal, 25% meat and bone scrap, and 15% menhaden fish meal. By analysis (N × 6.25) it contained 48.31% protein.

[3] The percentage composition of mineral mixture no. 3 was as follows: 40.0 ground limestone, 31.2 steamed bone meal, 25.0 iodized NaCl, 2.0 $MgCO_3$, 1.0 $FeSO_4 \cdot H_2O$, 0.56 $MnSO_4 \cdot H_2O$, 0.10 $CoCl \cdot 6H_2O$, 0.1 $CuSO_4$, 0.04 $ZnCO_3$.

[4] Guaranteed to supply 2 gm. procaine penicillin and 3 mg. vitamin B_{12} (L. L. elution assay) per pound.

[5] In drylot additions per lb. of ration were: 1.4 mg. riboflavin, 5.0 mg. calcium pantothenate, 5.0 mg. niacin, and 1.0 gm. choline chloride powder (25% choline chloride). On pasture, the vitamin mixture contained, in mg. per lb., not less than 2000 riboflavin, 4000 pantothenic acid, 9000 niacin, 10,000 choline chloride and 60 folic acid.

consumption records were obtained, in the same manner as with the drylot growth test.

On pasture, 14 weanling Chester White pigs, weighing an average of 27 lb., were selected from 3 litters and paired on the basis of litter, weight and general condition. Six of the 7 pairs were littermates. The pairs were divided at random between 2 lots offered, free choice, the same yellow corn, either ground or shelled, plus the same protein supplement, as that used in the pasture growth test. The supplement included the same addition of minerals, vitamins and an antibiotic as the 14-percent-protein ration in the pasture growth test. In addition, mineral mix 3 was supplied free choice.

Each lot occupied a one-half acre tract of the same pasture and was fed and watered, and its liveweight and feed consumption records were obtained, in the same manner as with the pasture growth test.

Results

Growth Tests

The results of the drylot and pasture growth tests are presented in table 2. In drylot, statistical treatment of the data for phase 1 indicated that the average daily gain was significantly lower at the 10 per-

TABLE 2. PERFORMANCE OF PIGS FED VARIOUS LEVELS OF PROTEIN ON DRYLOT AND PASTURE

Level of Protein, Percent	8	10	12	14	16
Drylot					
Av. initial wt., lb.		31.3	31.4	31.0	30.9
Phase 1					
Av. daily gain, lb.[1]		0.88	1.22	1.30	1.35
Av. daily feed per pig, lb.		3.47	3.95	4.10	3.97
Av. feed per lb. gain, lb.		3.94	3.24	3.15	2.94
Phase 2					
Av. daily gain, lb.[2]		0.97	1.35	1.37	1.40
Av. daily feed per pig, lb.		4.41	4.96	5.17	5.02
Av. feed per lb. gain, lb.		4.55	3.67	3.77	3.59
Pasture					
Av. initial wt., lb.	29.6	29.3	29.8	29.0	
Phase 1					
Adjusted av. daily gain, lb.[3]	0.98	1.12	1.35	1.43	
Standard error	±0.04	±0.04	±0.04	±0.06	
Av. daily feed per pig, lb.	2.88	2.98	3.57	3.58	
Av. feed per lb. gain, lb.	2.94	2.66	2.64	2.50	
Phase 2					
Adjusted av. daily gain, lb.[3]	1.12	1.21	1.47	1.48	
Standard error	±0.04	±0.03	±0.03	±0.04	
Av. daily feed per pig, lb.	3.60	3.81	4.76	4.38	
Av feed per lb. gain, lb.	3.22	3.14	3.25	2.95	

[1] Least significant difference at 5% level is 0.09 lb.
[2] Least significant difference at 5% level is 0.07 lb.
[3] Gains were adjusted for influence of initial on final weight.

cent protein level than at the 12, 14 and 16 percent levels, and that at 12 percent was significantly lower than at 16 percent but not 14 percent. For phase 2 the average daily gain at the 10-percent level was significantly lower than at the 12, 14 and 16-percent levels. The feed requirement per pound of gain decreased somewhat as the protein level was increased.

On the pasture growth test 2 pigs were removed from the 14-percent-protein level for causes not related to treatment. The feed consumption

records were adjusted by removing the pro rata share of the missing pigs, and the missing data were taken into account by means of dummy variates. Statistical analysis of the rate of gain data for the pasture growth test indicated that a straight line will fit the data for

TABLE 3. PROTEIN SELECTION RESULTS, DRYLOT AND PASTURE

Items	Drylot Selection Test		Pasture Selection Test	
	Protein Supplement Fed[1] Free Choice with		Protein Supplement Fed[2] Free Choice with	
	Ground Corn	Shelled Corn	Ground Corn	Shelled Corn
Number of pigs	9	9	7	5[3]
Av. initial wt., lb.	29.9	28.9	26.3	28.6
	Beginning to 100 lb.			
Protein level selected, %	13.7	15.3	10.8	10.8
Av. daily gain, lb.	1.24	1.23	1.15	1.01
Feed per lb. gain, lb.				
Corn	2.43	2.14	2.97	2.44
Supplement	0.58	0.70	0.27	0.23
Total	3.01	2.84	3.24	2.67
	100 to 200 lb.			
Protein level selected, %	10.9	12.7	10.0	9.9
Av. daily gain, lb.	1.50	1.47	1.70	1.64
Feed per lb. gain, lb.				
Corn	3.45	3.19	3.97	3.36
Supplement	0.38	0.41	0.26	0.21
Total	3.83	3.60	4.23	3.57

[1] Calculated protein percentage of supplement mixed with minerals, vitamins and antibiotic=38.04.
[2] Calculated protein percentage of supplement mixed with minerals, vitamins and antibiotic=42.44.
[3] Two pigs were lost from this lot for causes not related to treatment.

phase 1 quite well. The analysis for phase 2 indicated that a straight line may fit the data, although the gains on the 14-percent level were below, but not significantly below, the value estimated by extrapolation from the lower levels, suggesting that the minimum protein allowance may be between 12 and 14 percent.

Protein Selection Tests

The results of the drylot and pasture protein selection tests are presented in table 3. In drylot, the pigs on ground corn generally ate more corn and less supplement throughout the test than did the pigs on

shelled corn. On either shelled or ground corn, progressively lower levels of protein were selected as the size of the pigs increased.

On pasture, feed consumption records were adjusted by removing the pro rata share of two pigs lost from the shelled corn lot for causes not related to treatment. In general the pigs on ground corn ate more corn and slightly more supplement than those in the shelled corn lot. Unlike the pigs in drylot, the trend to select lower protein levels as size increased was not evident on pasture. Also the levels selected on ground corn and on shelled corn were almost the same.

Discussion

The results obtained by some investigators differ in some respects from the results obtained in this study and from each other. This is understandable, however, as the conditions of the different trials vary, from a little in some cases, to considerably in others.

The requirement of pigs for protein as reported by Mitchell and Hamilton (1935) are higher than those observed in this study. They concluded that the pig from 40 to 100 lb. required for maximum growth a ration containing more than 17 percent protein, whereas at heavier weights a ration containing 15 percent of total protein is somewhat more than adequate, but their conclusions were based on experiments in which different conditions existed than those in the tests reported here. Instead of group feeding, they used pair feeding in which the feed intake was limited to that quantity eaten by the poorer eating pig of the pair. This was undoubtedly one cause of the much lower rates of gain obtained in their experiment than in this study. They used nitrogen retention and carcass composition in addition to rate of growth as criteria.

As to their relatively lower rates of gain, Mitchell and Beadles (1952), based on their work with rats, concluded that the indicated minimum level of dietary protein required for the most rapid growth was not a function of the rate of food consumption within wide limits. Work with pigs by Becker, Ullrey and Terrill (1954) supported this. However, Crampton (1939) believed that in estimating protein requirements it is necessary to consider the level of food intake. Mitchell and Hamilton also used rations in their study which were different from those in this study. The makeup of their rations was as follows: in one case corn was mixed with a supplement of tankage, linseed oil meal and alfalfa meal, and in another case corn was mixed with a supplement of tankage, soybean oil meal and alfalfa meal, both supplements

in the proportion of 2:1:1. In a third case a special beef meal was used with corn and alfalfa meal. All of their rations contained salt, bone meal being added in proportions varying inversely with that of the supplemental mixture so that the influence of the latter would be restricted to its protein content. The energy value of comparable rations was equalized with corn oil. No antibiotic or crystalline vitamin addition was made.

Using rate of gain as a criterion, the protein concentration in the ration required for maximum gains of pigs determined by Carroll and Burroughs (1939) was also higher than that found in this study. They reported the requirement to be 18 to 20 percent for the 50 to 75 lb. pig, 15 percent for the 75 to 150 lb. pig, and 12 percent for the heavier pig. However, their conditions also differed in several respects from those in the present study or in the study by Mitchell and Hamilton. Instead of feeding for the same length of time on trial they fed to the same final weight. Their supplement to ground corn was a special beef meal and 4 percent alfalfa meal. Their mineral additions consisted of 0.5 percent salt plus the addition of bone meal to the low and medium protein ration to make them equal in mineral content to the high. No other mineral was used and no vitamin or antibiotic was added. They group fed in all cases.

Keith and Miller (1940) used nomographs based on the economy of gains in their tests and recommended 22 percent protein from 40 to 70 lb., 17 to 20 percent from 70 to 80 to 125 lb., and 15 to 17 percent from 125 to 200 lb. As in the test reported here, they fed pigs for the same length of time, rather than to the same final weight, and they used rate of gain as a criterion. However, instead of group feeding, they equal fed, with the feed intake being adjusted to the pig eating the least. Their supplement to corn was tankage, soybean oil meal and alfalfa meal. The soybean oil meal and tankage were increased equally to obtain the protein levels desired. Four percent alfalfa meal was used in one case; 10 percent was used in another case. Salt was added at the rate of 0.5 percent of the ration. There was no vitamin or antibiotic addition.

Woodman and Evans (1948), using very different rations, made an extensive study of the protein needs of pigs in a series of trials. They used controlled feeding at arbitrary rates. Their rations consisted of barley meal and weatings (mixture of bran and shorts) plus 2 percent alfalfa meal, mixed with a supplement composed of blood meal, dried separated milk and soybean oil meal, or with a supplement composed of white fish meal at varying percentages. Their mineral addition con-

sisted of 1 part salt and 3 parts ground chalk varied according to the diet. No trace mineral, vitamin or antibiotic addition was made. Their conclusions, converted to calculated protein percentages, were that rations which contained 17.5 percent protein to 90 lb. and 13.4 percent from 90 to 200 lb. were as good as rations containing 19.3 and 15.6 percent for those weights.

Becker, Lassiter, Terrill and Norton (1954) obtained results very similar to those reported here using corn-soybean oil meal and corn-fish meal rations. Their diets included the same addition of trace minerals, vitamins and antibiotic as the drylot ration in this study. Their soybean oil meal diet did include steamed bone meal in addition to the other mineral additions. They likewise fed to the same length of time on trial rather than to the same final weight. They utilized equal and full feeding individually rather than group feeding. Their conclusions were that from 40 to 100 lb., with corn-fish meal rations, pigs require a minimum of 16 percent protein, but in corn-soybean oil meal rations 14 percent is adequate for this growth period. They concluded that from 100 to 200 lb. a minimum of 12 percent protein was needed with either soybean oil meal or menhaden fish meal.

Self feeding to the same average lot weight, rather than to the same date has been used by a number of investigators. They employed either different supplements, different antibiotics, different vitamin supplementation, or they worked toward different objectives. Hoefer *et al.* (1952 used 2 percent fish solubles in corn-soybean oil meal rations with and without 5 mg. terramycin per pound of ration and concluded that terramycin did not affect the pig's requirement for protein. Their results, however, showed that 15 followed by 12 percent protein was as good as 18 followed by 15 percent protein for the pig from 25 to 100 and 100 to 200 lb. liveweight, respectively. Catron *et al.* (1952) with fortified corn-soybean oil meal rations determined that 10 mg. aureomycin per pound of ration reduced the protein required about 2 percent at the different weight ranges. They concluded that for pigs from weaning to 75 lb., 75 to 150 lb. and 150 to 200 lb., the requirement without aureomycin was 16–13–10, respectively, and with aureomycin was 14–11–8, respectively.

Wallace *et al.* (1954) found no clear evidence that aureomycin enhanced protein utilization. However, they differed from Catron *et al.* in that they incorporated 5 percent alfalfa meal into their corn-soybean oil meal rations, and added aureomycin at a rate 20 times as high as Catron *et al.* Their B-vitamin additions were from more than double to more than 6 times those of Catron *et al.* They reported that 14.3

percent protein was as good as 17.6 or 20.9 for pigs from about 40 lb. to about 190 lb. liveweight.

Burnside *et al.* (1954) used 7 percent alfalfa meal in their corn-soybean oil meal rations with the addition of no vitamin other than B_{12}. They added B_{12} and aureomycin at about the same rate as Catron *et al.* (1952). They felt that aureomycin, alone or with B_{12} increased the protein utilization to the extent that 17–13.7–11.5 percent was as good as 21–18.2–15 percent from weaning to 70 lb., 70 to 125 lb., and 125 to 213 lb. respectively.

Summary

The effect of protein level on growth has been compared with the protein levels selected by pigs offered the same diets as corn and protein supplement free choice. Comparison of the conditions of this study with the conditions of the studies of others emphasize the variations that may be applied to controlled experiments studying essentially the same question, and lead to the conclusion that minimum protein allowances for growing swine are not fixed quantities and may be stated precisely only by listing the specific conditions to which the precise allowances apply.

Under the conditions of these tests, in drylot, the minimum protein allowance appears to be 14 to 16 percent from weaning to 100 lb. When the same protein level is fed for the entire growth period from weaning to 200 lb., the minimum allowance appears to be 12 to 14 percent.

On alfalfa pasture, according to the growth test the minimum protein allowance appears to be 12 to 14 percent from weaning to 200 lb., although the higher protein level appears advisable for pigs under 100 lb. Pigs on pasture that had corn and supplement available, free choice, selected protein levels at 10.8 percent from weaning to 100 lb. and 10.0 percent from 100 to 200 lb.

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SUPPLEMENTARY PROTEIN AND THE RESPONSE OF THE PIG TO ANTIBIOTICS

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A REVIEW of the literature concerning the use of antibiotics in swine nutrition has been published by Braude, Wallace, and Cunha (1953). These reviewers have discussed several factors which have been associated with the growth stimulation of an antibiotic.

With regard to the nature of the supplementary protein there are many reports which show that the growth response of the chick or pig to an antibiotic is less when diets containing fish meal are fed rather than all-vegetable diets. Using an antibiotic and vitamin B_{12} supplement Burnside *et al.* (1949) reported that the supplement supplied an unknown factor or factors that increased the feeding value of peanut meal and soybean oil meal so that these plant protein supplements were equal to fish meal for the pig. Braude *et al.* (1953) obtained a small response to antibiotics on a well-balanced diet containing high quality white fish meal and a considerable response on a diet containing vegetable protein only. Similar results have been reported by Robinson *et al.* (1952).

With the chick Matterson *et al.* (1951) concluded that the growth response of chicks obtained by the addition of antibiotics was not as great when fish meal was included in the diet. The recent studies of Branion and Hill (1953) have indicated that fish meal contains a factor which lowers the percent growth response of the chick to an antibiotic.

The objective of the studies reported here was to compare soybean oil meal and menhaden fish meal as supplementary proteins upon the response of the pig to a single or to a mixture of antibiotics. In addition, the nature of the nutritive deficiency of the corn-menhaden fish meal diet was investigated.

Experimental

Experiment 1

Thirty-six Duroc pigs of approximately weaning age were used in this study. The pigs were raised to a weaning age of 8 weeks by sows fed

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adequate rations in drylot. Twelve outcome groups were made up of littermates, or pigs of similar ancestry, on the basis of sex, thriftiness and initial weight, and the outcome groups were paired on the basis of weight. Within the outcome groups the pigs were assigned at random to the antibiotic treatments which are presented in table 2. In addition, from each outcome group pair one group was chosen at random to receive soybean oil meal and the remaining group was fed menhaden fish meal as the source of supplementary protein.

The ingredients of the diets used are presented in table 1.

TABLE 1. PERCENTAGE COMPOSITION OF DIETS FED

Ingredients	Corn-Soybean Oil Meal	Corn-Menhaden Fish Meal
Ground yellow corn	73.1	81.7
Solvent soybean oil meal	23.6	....
Menhaden fish meal	....	16.0
Steamed bone meal	1.0	....
Mineral mixture no. 3 [1]	2.0	2.0
Vitamin A and D concentrate (4000A, 750D)	0.2	0.2
Vitamin B_{12} supplement [2]	0.1	0.1
Vitamin mixture no. 36 [3]	+	+
Crude protein (N×6.25) [4]	18.1	17.5
Tryptophan [4]	0.17	0.10
Nicotinic acid (calculated), mg./lb.	15.2	17.4

[1] The percentage composition of mineral mixture No. 3 was as follows: ground limestone, 40.0; steamed bone meal, 31.2; iodized NaCl, 25.0; $MgCO_3$, 2.0; $FeSO_4 \cdot XH_2O$, 1.0; $MnSO_4 \cdot H_2O$, 0.56; $CoCl_2 \cdot 6H_2O$, 0.10; $CuSO_4$, 0.1 and $ZnCO_3$, 0.04.

[2] Guaranteed to contain 6 mg. of vitamin B_{12} per pound.

[3] Additions to each pound of diet were as follows: riboflavin, 1.4 mg.; calcium pantothenate, 5.0 mg.; nicotinic acid, 5.0 mg.; and choline chloride powder (25 percent choline chloride), 1.0 gm.

[4] By analysis of diet used in Experiment II.

Each treatment group was kept on concrete lots which were provided with adequate shelter. The pigs were self-fed *ad libitum*, and an adequate water supply was maintained. The pigs were weighed individually at weekly intervals and records of feed consumption were kept. The duration of the test was 8 weeks.

The growth data were treated statistically by analysis of variance as outlined by Snedecor (1946).

Experiment II

Thirty-two Hampshire pigs weaned at 8 weeks from sows fed adequate diets on alfalfa pasture were used for this test. The pigs were allotted to the treatment groups at random from outcome groups formed

on the basis of ancestry, age and initial weight. The treatments are presented in table 4. Diets, feeding technic, management, and statistical analysis were the same as described above.

TABLE 2. PERFORMANCE OF PIGS FED CORN-SOYBEAN OIL MEAL AND CORN-FISH MEAL DIETS WITH AND WITHOUT A SINGLE OR A MIXTURE OF ANTIBIOTICS
(Experiment I)

Lot No.	1	2	3	
Treatment	Control	Chlor-tetracycline HCl (6 mg./lb.)	Chlor-tetracycline HCl Procaine Penicillin Streptomycin (2 mg./lb. of each)	Av.
Av. initial wt., lb.				
Corn-soybean oil meal	35.3	34.2	34.0	34.5
Corn-fish meal	35.2	34.8	35.2	35.1
Av.	35.2	34.5	34.6	
Av. final wt., lb.				
Corn-soybean oil meal	105.5	111.5	114.0	110.3
Corn-fish meal	75.3	90.7	109.2	91.7
Av.	90.4	101.1	111.6	
Av. daily gain, lb.				
Corn-soybean oil meal	1.25	1.38	1.43	1.35
Corn-fish meal	0.72	1.00	1.32	1.01
Av.	0.99	1.19	1.38	
Av. daily feed intake, lb.				
Corn-soybean oil meal	3.47	3.89	4.05	3.80
Corn-fish meal	2.46	2.91	3.79	3.05
Av.	2.96	3.40	3.92	
Feed per lb. gain, lb.				
Corn-soybean oil meal	2.77	2.82	2.84	2.81
Corn-fish meal	3.43	2.92	2.87	3.02
Av.	3.01	2.86	2.85	

Results

The growth and feed data for Experiment I are presented in table 2. As indicated in the analysis of variance in table 3 there was a significant (P less than 0.05) interaction of antibiotic with the supplementary protein. Antibiotics with soybean oil meal as the supplement produced growth responses out of accord with those obtained with the corn-fish meal diet. With the corn-soybean oil meal diet chlortetracycline and the antibiotic mixture produced gain increments of 10.4 and 14.4

percent, respectively; whereas, these figures with the corn-fish meal diet were 38.9 and 83.3 percent, respectively. In regard to efficiency of feed conversion, antibiotics failed to exert any influence with soybean oil meal as the protein supplement, but in the case of fish meal, either type of antibiotic addition produced approximately equal improvement. The antibiotic mixture was superior to chlortetracycline alone with either protein, although the superiority of the mixture was more evident with the corn-fish meal diet.

TABLE 3. ANALYSIS OF VARIANCE OF AVERAGE DAILY GAINS IN EXPERIMENT I

Source of Variance	d/f	Variance
Antibiotics (A)	2	0.4566
Type of protein (P)	1	1.0540*
Pair groups (G)	5	0.1733
A×P	2	0.1388*
A×G	10	0.0337
P×G	5	0.1127*
A×P×G	10	0.0264

* P less than 0.05.

In the absence of antibiotics there was a marked difference in the performance of pigs fed the two sources of supplementary protein. In this case, the pigs fed soybean oil meal gained 73.6 percent faster; however, in the presence of the antibiotic mixture pigs fed the corn-soybean oil meal diet gained only 8.3 percent faster. With the single antibiotic the difference in gains on the two supplementary protein was an intermediate value.

A statistically significant interaction occurred between pair groups and type of protein. In every case, the performance of the trio which was fed fish meal gained at a slower rate than the paired-trio fed soybean oil meal, but the magnitude of this difference within the pair groups varied considerably and this variation was not associated with initial weight.

The growth and feed data for Experiment II are presented in table 4.

In general the performance in the latter test confirmed the results of the previous experiment in regard to the type of supplementary protein. Although the difference in daily gains on the two types of protein failed to attain the necessary difference value of 0.34 lb., the performance of pigs fed the corn-soybean oil meal diet and the corn-fish meal diet plus tryptophan was about the same. The addition of fish

soluble solids did not improve the rate or efficiency of gains of pigs fed the fish meal diet.

Pigs fed the corn-fish meal diet in the absence of antibiotics exhibited rough scaly skin and an unkempt hair coat. There was some evidence of a generalized brownish exudate over the body, however, it never became pronounced. Diarrhea was never observed.

TABLE 4. THE SUPPLEMENTARY VALUE OF TRYPTOPHAN AND FISH-SOLUBLE SOLIDS TO A CORN-MENHADEN FISH MEAL DIET
(Experiment II)

Lot No.	1	2	3	4
Treatment—Diet	Corn-Soybean Oil Meal	Corn-Menhaden Fish Meal		
Supplement			0.05% DL-tryptophan	2.0% Fish Soluble Solids [1]
Number of pigs	8	8[2]	8[2]	8
Av. initial wt., lb.	41.1	39.8	40.4	40.6
Av. final wt., lb.	99.2	78.4	99.0	80.6
Av. daily gain, lb.[3]	1.04	0.74	1.16	0.71
Av. daily feed per pig, lb.	3.18	2.91	3.97	3.22
Feed per lb. gain, lb.	3.06	3.90	3.44	4.51

[1] Replaced an equal quantity of menhaden fish meal.
[2] One pig removed from each lot for reasons not associated with treatment and data for these pigs are included.
[3] Necessary difference value (5%) = 0.34 lb. daily.

Discussion

The magnitude of the growth response to antibiotics did vary with the type of supplementary protein employed in the studies reported here. The response to antibiotics was less with an all-vegetable diet than with a diet in which menhaden fish meal provided the supplementary protein. Previously in the pig Braude *et al.* (1953) and Burnside *et al.* (1949) have reported that greater growth responses were obtained when soybean oil meal was used as the supplementary protein. Similar findings in the chick have been observed by Branion and Hill (1953) and also by Matterson *et al.* (1951).

Although the corn-fish meal diet was formulated to contain ample protein for the weanling pig, the evidence suggests that the diet did not supply sufficient tryptophan to satisfy the requirement. Braude *et al.* (1953) in their review have concluded that pigs fed a ration with better over-all nutrient balance will respond less to antibiotic supplementation than with a poorer ration. Particularly from the standpoint of amino

acid balance the data presented here supports this view. Certainly there was no evidence to confirm the conclusion of Branion and Hill (1953) that fish meal contains an unidentified factor which lowers the percent response to an antibiotic.

In previous studies regarding the interaction of antibiotics with the supplementary protein there was no indication that the fish meal diets were deficient in tryptophan. For instance, the studies of Burnside *et al.* (1949) employed diets which contained 22.78 percent protein and it is unlikely that such a diet would be deficient in tryptophan.

The results raise the question as to the ability of antibiotics to spare the need for tryptophan by the pig. Jones and Combs (1951) have reported that antibiotics exert a tryptophan-sparing action in the chick. Likewise, Sauberlich (1954) has recently suggested the occurrence of a tryptophan-sparing action by antibiotics in the rat. If we arbitrarily define a sparing effect as the ability of an antibiotic to improve growth on a tryptophan deficient diet, then the results presented here would suggest such an effect. In addition, the mixture of antibiotics exerts a greater sparing action than chlortetracycline alone. At any rate, the data agree with the earlier studies of Becker *et al.* (1954) that the tryptophan requirement of the weanling pig would appear to be no greater than 0.15 percent of the diet. Although 0.10 percent tryptophan was insufficient to support normal growth an addition of 0.05 percent DL-tryptophan increased growth rate to that obtained on the corn-soybean oil meal diet which contained 0.17 percent tryptophan.

Previously Becker *et al.* (1953) reported that a mixture of antibiotics made up of oxytetracycline, procaine penicillin, bacitracin, and sometimes streptomycin was superior to oxytetracycline alone when fed to the pig at 16 mg. per pound of diet. Particularly with the corn-fish meal diet, a mixture of chlortetracycline, procaine penicillin and streptomycin was superior to chlortetracycline alone when fed at a more practical rate of 6 mg. per pound of diet.

Summary

Soybean oil meal and menhaden fish meal were used to investigate the effect of the supplementary protein upon the response of the pig fed a single or a mixture of antibiotics. At a rate of 6 mg. per pound of diet either chlortetracycline or a mixture of equal parts chlortetracycline, procaine penicillin, and streptomycin increased the rate of gain of weanling pigs fed a corn-soybean oil meal diet and the rate and efficiency of gain of those fed the corn-fish meal diet. The magnitude of

the growth response from antibiotics varied with the supplementary protein, it being considerably greater with menhaden fish meal as the supplementary protein. The growth increment produced by the mixture of antibiotics was superior to chlortetracycline alone, particularly with the corn-fish meal diet. In the absence of antibiotic the corn-fish meal diet which contained 0.10 percent tryptophan was significantly improved by the addition of 0.05 percent of DL-tryptophan.

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THE EFFECT OF GRADED LEVELS OF ALFALFA AND AUREOMYCIN UPON GROWING-FATTENING SWINE [1]

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EARLY work (Ellis *et al.*, 1942; Evvard 1929, Oliver and Potter, 1930; Headley, 1932; Fargo *et al.*, 1941) indicated that rations for growing-fattening swine should not contain over 15 percent alfalfa or 5–6 percent fiber for optimum growth. Forbes and Hamilton (1952) found that 125 lb. pigs could digest 42.5 and 49.7 percent of the crude fiber and cellulose, respectively in a ration containing 23.6 percent alfalfa. More recently Bohman *et al.* (1953) and McCormick and Kidwell (1953) have shown that hogs could use rations containing up to 50 percent alfalfa and gain at the rate of 1.3–1.7 lb. daily for the entire growing fattening period.

The value of antibiotics in swine rations has been adequately reviewed by Braude *et al.* (1953). This summary shows that chlortetracyline (Aureomycin) increased the daily gain of growing-fattening swine 35 percent more than control animals in experiments conducted throughout the United States. Terramycin in a ration containing 20 percent alfalfa had no effect upon rate of gain of swine (Bohman *et al.*, 1953). Neuman *et al.* (1951) and Bell *et al.* (1950) have indicated that antibiotics may adversely affect the beneficial symbiotic bacteria in ruminants. Hogs must also depend upon intestinal bacteria for cellulose and fiber digestion. Since Forbes and Hamilton (1952) decreased the digestibility of cellulose and crude fiber by swine with the addition of sulfathalidine, it seems that antibiotics may play a similar role. This could be reflected by a reduced rate of gain or no difference in rate of gain when a high roughage ration is fed with antibiotics.

This experiment was conducted (1) to obtain additional information on the use of high roughage rations by swine and (2) to determine the effect of aureomycin on swine fed graded levels of alfalfa.

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Experimental Procedure

Seventy-two Minnesota #1 × Duroc weanling pigs were divided into 8 groups according to weight, sex and litter and allotted at random to experimental treatments. These treatments consisted of 4 levels of alfalfa (0, 10, 30 and 50 percent of the ration) with and without aureomycin (10 mg. per lb. of feed). The rations containing the 0 levels of alfalfa were composed of 75.3 percent ground milo and 24.7 percent

TABLE 1. CHEMICAL COMPOSITION OF RATIONS

		Composition of Dry Matter					
Percent Alfalfa	Dry Matter	Crude Protein	Ether Extract	Crude Fiber	NFE	Ca	P
Weaning to 120 lb.	%	%	%	%	%	%	%
0	90.4	23.8	4.7	4.3	59.5	2.48	1.24
10	90.4	22.6	4.8	6.9	58.0	2.54	1.13
30	91.1	22.3	4.9	12.6	51.4	2.48	0.99
50	90.8	21.7	4.0	17.6	47.2	2.54	0.87
120–200 lb.							
0	90.9	19.6	4.0	3.6	66.8	1.69	0.97
10	91.0	19.5	5.1	7.0	62.6	1.73	0.86
30	90.0	19.2	4.3	13.0	57.0	1.69	0.67
50	91.6	18.7	3.4	17.1	53.0	1.90	0.59

protein supplement. The protein supplement contained 40 percent cottonseed meal and 30 percent of both meat scraps and fish meal. For the high roughage rations, alfalfa was added to this mixture and the milo and protein supplement were adjusted to equalize the level of protein in all rations. After the pigs reached 120 lb. liveweight, the protein content of the ration was reduced approximately 3 percent by adjusting the percentage of milo and protein supplement for all groups. The chemical compositions of the rations are given in table 1.

The aureomycin was premixed with milo and then included with the other ingredients. Additional vitamin A, 1,157 I.U. per lb. of feed, was added in the form of gelatin covered pellets of vitamin A acetate [4] to the rations without alfalfa. All rations were pelleted and self fed; automatic waterers were used. All animals were weighed at bi-weekly intervals. On the first weigh day after a pig reached 200 lb. liveweight it was removed from the experiment and slaughtered by a local meat packing company. Slaughter data were obtained on some of the pigs at this time. Dressing percentage was calculated as the percent hot carcass weight of the non-shrunk liveweight. Depth of back fat was determined as the average of three measurements taken opposite the

[4] Supplied by the courtesy of Chas. Pfizer and Co., Inc., Brooklyn, N. Y.

first and last rib and the last lumbar vertebra. Carcass length was measured from the front edge of the first rib to the aitch bone. The shoulder, ham, loin, bacon belly and fat back were cut out by one individual and calculated as percent of the hot carcass. The weight of the heart, lungs, liver, spleen, esophagus, stomach, small intestine, and large intestine was obtained. All viscera were weighed free from blood, digestive tract fill and superficial fat.

TABLE 2. THE EFFECT OF AUREOMYCIN AND GRADED LEVELS OF ALFALFA UPON GROWTH AND FEED UTILIZATION

	Aureomycin				Controls			
Level of Alfalfa, %	0	10	30	50	0	10	30	50
No. of pigs	9	9	9	9	9	9	9	8[1]
Av. days of experiment	82	97	120	167	96	104	125	172
Average, lbs.								
Initial weight	38	37	37	37	37	37	38	33
Final weight	203	213	209	203	212	202	207	206
Daily gain	2.07*	1.85	1.45	1.01	1.82	1.72	1.37	1.01
Daily feed consumption, lb.	6.0	6.1	5.5	5.2	5.6	5.7	5.4	5.4
Feed per lb. gain, lb.	2.98	3.35	3.87	5.24	3.06	3.57	4.04	5.40
Concentrate per lb. gain, lb.	2.98	3.01	2.71	2.62	3.06	3.21	2.83	2.70

[1] One pig died from non-experimental causes.
* Difference between alfalfa levels is significant at P .01.

Results and Discussion

The results of the growth data are given in table 2. As the level of alfalfa in the ration increased, the average daily gain decreased significantly (P less than .01) and consequently the days on experiment increased. These findings are in agreement with previous work at this station (Bohman *et al.*, 1953; Kidwell and McCormick, 1953). However, in this experiment, the differences between alfalfa levels were more pronounced. The quality of alfalfa was not as good as that used in previous experiments. This was confirmed by limited studies with rats. Rats fed rations containing 50 percent of this alfalfa failed to grow while rats fed equal quantities of alfalfa used in previous experiments made fair growth. When alfalfa was substituted for 50 percent of the basal ration, Crampton *et al.* (1954) found that the growth rate of swine subsequently decreased and the daily feed consumption increased. In this study daily feed consumption was less for the hogs fed high levels of alfalfa. Part of the lowered rate of gain in the higher alfalfa rations could be attributed to a decreased daily feed in-

take, but the differences in daily feed intake are not adequate to account for the differences in daily gain. The rate of gain was inversely proportional to the crude fiber level in the rations and this appears to be the factor exerting the greatest influence upon the experimental results. Axelsson and Eriksson (1953) have stated that for growth the optimum level of crude fiber in the dry matter of the rations averaged 6.57 percent, but 7.26 percent fiber was required for the maximum efficiency of metabolizable energy. The data presented in this paper do not support this view. In this study, the greatest gain and best economy of gain was obtained on rations containing 4.30 percent crude fiber. If an optimum level of crude fiber in growing swine rations exists it is probably lower than the level stated by Axelsson and Eriksson (1953) or the source of crude fiber influences any postulated optimum level for swine. Forbes and Hamilton (1952) have determined that the source of crude fiber is an important factor affecting the digestibility and availability of nutrients from various cellulosic substances. Cellulose in alfalfa meal was more digestible than the same material in wheat straw. The source of fiber in Axelsson and Eriksson's (1953) study was wheat straw. Lepkovsky *et al.* (1950) have indicated that a non-fibrous factor was responsible for the growth depressing effect observed when high levels of alfalfa were fed to growing chicks. In additional studies, Hill and Dansky (1951) fed oat hulls up to 40 percent of the ration and found that these high levels of fiber had little effect upon the growth of poultry as expressed by weight gains. Peterson *et al.* (1954) obtained similar results when graded levels of cellu flour were fed. In these last two experiments, an increased feed intake compensated for the higher fiber rations. This would indicate that the source of fiber or the presence of a growth inhibitor for swine in alfalfa may be responsible for the difference in response of swine in Axelsson and Eriksson's (1953) experiments and the present study. However, other factors such as type of hog may also be important. Teague and Hanson (1954) added fiber to swine rations in the form of Ruffex at levels that equaled and exceeded those in the current study and found only a slight depression of growth rate and feed utilization which further substantiates the above hypothesis.

In this study as the level of alfalfa in the ration increased, the amount of feed required per 100 lb. gain also increased. Except for the pigs fed the highest level of alfalfa, less than 4 lb. of feed were required per lb. of gain. Still, pigs fed rations containing 50 percent alfalfa consumed less concentrate per lb. of gain than any of the other groups.

When aureomycin was added to the rations, the rate of gain significantly increased (P less than .05). The pigs receiving aureomycin gained 1.60 lb. daily while the controls averaged 1.48 lb. As the level of alfalfa in the ration increased, the difference in rate of gain between the pigs receiving aureomycin and the controls decreased. At the 50 percent alfalfa level, the pigs receiving aureomycin gained at the same rate as the controls. This interaction was not statistically significant. Braude *et al.* (1953) in their review noted that the percentage increase due to antibiotics was inversely proportional to the rate of gain. In this study the greatest difference between the controls and the antibiotic-fed animals occurred on the rations producing the most rapid rate of gain, Monson *et al.* (1954) found that aureomycin stimulated the production of folic acid in the intestines of poultry by favoring the establishment of the coliform organisms that synthesize this vitamin. It is possible that this mechanism may account for the vitamin sparing action with other vitamins as well. If alfalfa added one or more nutrients to the ration at sub-optimum levels except for the highest alfalfa level, it may explain the decreasing response to aureomycin with increasing alfalfa levels. The slower growth rate of hogs on the higher alfalfa levels might be attributed to some other dietary factor such as level of fiber or a growth depressing factor. Experiments are now underway to determine if the digestibility of these rations played a role in regard to the results obtained.

Aureomycin increased the efficiency of feed utilization as measured by the pounds of feed per pound of gain for each alfalfa level. Even when the rate of gain was equal (50 percent alfalfa level) the pigs supplemented with aureomycin were more efficient.

The effect of the alfalfa levels on carcass measurements is shown in table 3. Aureomycin had no effect upon any of the observations, therefore the data presented are the combined average of treatments with and without antibiotics.

As the level of alfalfa increased, the dressing percentage, the depth of back fat, and the percent of bacon belly and fat back in the carcass significantly decreased while the percent of ham, shoulder and loin increased significantly. These data indicate that hogs fed high levels of alfalfa produce a leaner carcass with a much higher percentage of lean cuts at the expense of dressing percentage and fatter cuts. Leaner cuts are becoming more popular and since fats are used less for soaps, hogs fed higher levels of alfalfa approach the market demands more closely than hogs fed the conventional rations. Crampton *et al.* (1954) found similar results when rations for hogs from 115–200 lb. were

"diluted" with fibrous feeds. The more desirable and leaner hogs in his study were produced when fibrous feeds were added to more concentrated rations. Axelsson and Eriksson (1953) also observed that as the level of fiber increased from 4.8 to 9.6 percent, there was a tendency for an increased leanness in the carcass. However, they indicated that type played a more important role than rations in this respect. In a purified ration for hogs, Teague and Hanson (1954)

TABLE 3. THE EFFECT OF GRADED LEVELS OF ALFALFA ON CARCASS CHARACTERISTICS

Level of Alfalfa, %	0	10	30	50	L.S.D. P.05	L.S.D. P.01
Dressing percentage	74.6	73.0	69.6	68.1	2.4	3.2
Depth of back fat, in.	1.80	1.73	1.31	1.11	0.16	0.21
Carcass length, in.	29.6	29.8	30.0	30.3	n.s.	n.s.
Ham in carcass, %	15.7	16.2	17.7	18.9	0.8	1.1
Loin in carcass, %	14.1	14.7	14.6	15.9	0.8	1.1
Shoulder in carcass, %	14.0	14.2	14.9	15.5	0.5	0.7
Bacon belly in carcass, %	13.7	14.1	13.3	12.4	0.8	1.1
Fat back in carcass, %	11.3	10.4	6.9	5.1	1.1	1.4
Total lean cuts in carcass, %	43.8	45.1	47.2	50.3	2.1	2.9
Total fat cuts in carcass, %	25.0	24.5	20.2	17.5	1.3	2.3

n.s.=non significant.

increased the fiber level by adding Ruffex and found no correlations of the carcass characteristics with the amount of fiber fed. Hill and Dansky (1951) decreased the amount of body fat in growing chicks by increasing the level of fiber with oat hulls. This study shows that the leanness of a hog's carcass can be increased by increasing the level of fiber in the ration.

If animals are able to use high levels of fiber, it must be accomplished by bacterial enzymatic digestion. If a hog could become adapted to this type of digestion, it would appear that parts of the digestive tract would become enlarged. To check this hypothesis, the weights of many of the body organs were taken. Aueromycin had no effect on the size of any of the body organs measured.

As the level of alfalfa increased, the size of both the stomach and the large intestine increased. The stomach and large intestines of hogs fed rations containing 50 percent alfalfa was 22 and 32 percent heavier, respectively than hogs fed rations without alfalfa. This would indicate that the animal adapts itself to high levels of alfalfa intake by increasing the size of the organs that act as temporary food storage areas.

The small intestine was not affected, probably because of the speed in transit of feed through this organ. The increased size of the large intestine probably facilitates bacterial fermentation and the subsequent utilization of fiber by the hog. Sampson and Mussehl (1936) found that the length of the small intestine was increased when a high level of alfalfa was fed to cockerels.

Huang and McCay (1953) noted that terramycin seemed to favor the infestation of swine by ascarids. Aueromycin and alfalfa levels had no significant effect upon number of ascarids in this study. Most groups had a slight ascarid infestation.

Summary

Seventy-two Minnesota #1 × Duroc weanling pigs were alloted to 8 groups according to weight, breeding and sex. These animals were fed 4 levels of alfalfa meal (0, 10, 30, and 50 percent of the ration) with and without aureomycin (10 mg. per lb. of feed) in balanced, pelleted rations. All pigs were slaughtered as soon as possible after they reached 200 lb. body weight. Carcass data and organ weights were obtained. As the level of alfalfa in the ration increased, the rate of gain, dressing percentage, depth of back fat, and the percent of bacon belly and fat back in the carcass decreased significantly while the percent of shoulder, ham and loin in the carcass and the weight of the stomach and large intestine increased significantly. The pigs fed aureomycin gained significantly faster with less feed per pound of gain than the controls. None of the treatments had any effect upon the carcass length, number of ascarids, or weight of the spleen, liver, small intestine, lungs or heart. These data indicate that hogs may use up to 50 percent alfalfa in their rations and produce leaner carcasses than control pigs fed a conventional ration. Aureomycin accelerated the growth rate and decreased the feed required per pound of gain even at high levels of alfalfa feedings. Swine apparently adjust to a high level of alfalfa intake by enlarging the size of their digestive tracts where limited transitory feed storage takes place as indicated by the increased weights of the stomach and large intestine.

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THE EFFECT OF AUREOMYCIN ON GROWTH AND REPRODUCTION IN SWINE [1]

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THE widespread use of antibiotics for feeding swine during recent years has followed early work in that field by Cunha *et al.* (1949) and Jukes and coworkers (1950). Other reports from a number of workers have indicated that various antibiotics have different optimum levels at which they should be fed. For aureomycin, 5.0 to 10.0 mg. per lb. of feed were found to give satisfactory results during the growing-fattening period by Speer and coworkers (1950), Briggs and Beeson (1950 a and b), and Catron *et al.* (1951). Vestal and coworkers (1952) reported that 10.0 mg. of aureomycin per lb. of feed gave some improvement in reproductive performance, but that 4.5 mg. per lb. proved ineffective. Larson and Carpenter (1952), who used 10.0 mg. of aureomycin per lb. of feed on a group of sows for two successive farrowing periods, reported no apparent effect on reproduction although the sows fed the antibiotic supplement weaned more and heavier pigs.

Because of the increasing use of aureomycin in diets for breeding stock, a study was initiated in 1951 in order to secure information on the effect of continuous feeding through successive reproductive cycles and generations. Diets containing relatively high levels of aureomycin (50.0 and 100.0 mg. per lb. of feed) were included in this study along with a commonly accepted level (10.0 mg. per lb. of feed) in an attempt to accelerate or intensify the effect of continuous antibiotic feeding, and to study any possible toxic or undesirable effects of the high levels, as such, on growth and reproduction.

Materials and Methods

Sixteen young sows and gilts of predominantly Danish Landrace breeding were distributed among three lots. Each lot was started on its

[1] The data in this paper were taken from a thesis submitted by the senior author to the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science. The authors are indebted to J. C. Shaw at the University of Maryland for his advice and help on the experiment and to N. R. Ellis of the Animal and Poultry Husbandry Research Branch, U.S.D.A. for advice and for making facilities and animals available for the investigations.

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respective diet at the beginning of the breeding season in July 1951. When their litters were weaned, eight weanling gilts were selected from within each aureomycin treatment. These eight animals were carried through a growing-fattening period and one reproduction cycle on the same level of aureomycin that their respective dams had received. In the same manner, third generation pigs again were selected from within each aureomycin level to be continued on the same treatment. When they reached a weight of approximately 220 lb., the study was terminated.

The diet used had the following composition: ground yellow corn (grade No. 2) 67.5; soybean oil meal (solvent extracted) 25.0; alfalfa meal (17 percent protein, dehydrated) 5.0; complex mineral mixture [4] 1.5; steamed bonemeal 0.5; ground limestone 0.5 lb., plus irradiated yeast and calcium pantothenate to supply 100 I.U. of vitamin D and 600 mcg. pantothenic acid per lb. of feed. The protein level of this diet was approximately 18 percent and it was used during lactation and the growth period from weaning to an average weight of 90 lb. The corn-soybean oil meal ratio was adjusted to give a 15 percent protein level in the feed mixtures used during gestation and the growth period from 90 to 220 lb. The lactation diets in the second year contained 10 percent pulverized No. 2 white oats which replaced an equivalent amount of corn and soybean meal.

Each diet contained an aureomycin-vitamin B_{12} feeding supplement in an amount to provide 10.0 mg. of aureomycin per lb. of feed. Additional aureomycin in crystalline form was added to the 50.0 and 100.0 mg. treatments in order to reach the prescribed levels.

During all phases of the experiment the animals were kept in concrete-floored pens with access to outside paved yards. The sows were hand fed during the gestation period and gradually brought to full feed after farrowing. During the two growing-fattening periods the pigs were self fed. Biweekly weighings were made and feed consumption records kept by groups during the growth periods. Data were analyzed by use of the methods for analysis of variance and covariance given by Snedecor (1946).

Results and Discussion

The determination of possible effects on reproductive performance from feeding a commonly used (10.0 mg. per lb.) and higher levels

[4] Complex mineral mixture: iodized salt 30.8%; steamed bonemeal 31.7%; ground limestone 32.0%; magnesium sulfate 2.5%; ferrous sulfate 2.1%; copper sulfate 0.2%; manganese sulfate 0.5%; cobalt chloride 0.1%; and zinc oxide 0.1%.

(50.0 and 100.0 mg. per lb.) of aureomycin to successive generations of swine was the primary purpose of this study. Because no apparent symptoms of reproductive failure or growth depression were observed from a qualitative standpoint, statistical analyses were made on portions of the quantitative data in order to secure additional information from the study. These analyses were based on the combined data for the two years with preliminary within-year analyses being made on birth and weaning weights.

TABLE 1. SUMMARY OF DATA PERTAINING TO REPRODUCTION AND PRE-WEANING PERFORMANCE

Diet (mg. of Aureomycin per lb. of Diet)	Number of Litters	Average per Litter				
		Number Born		Birth Weight	Number Weaned	Weaning Weight
		Alive	Dead			
				lb.		lb.
		1951				
10	4	10.0	0.5	2.68	8.0	29.2
50	5	7.2	2.0	3.06	5.0	29.6
100	3	6.7	0.3	3.03	6.0	37.3
		1952				
10	3	7.0	2.7	2.66	5.7	31.8
50	4	9.5	0.8	2.13	6.3	35.7
100	7	8.7	0.3	2.51	7.3	26.8

The reproductive records for each year are given in table 1. There was no statistically significant effect on total fertility from feeding the high aureomycin levels, nor was any significant difference found in average numbers of pigs weaned per litter. There were apparently no adverse effects on survival of pigs born alive as inspection of the percentages of pigs surviving to weaning age revealed that the 100.0 mg. group had a higher survival rate (90.0 percent in 1951 and 83.6 percent in 1952) than either the 10.0 or 50.0 mg. groups.

On the basis of the combined two-years data, the differences in average birth weights for the 3 treatments were not statistically significant. The difference between years (1952 weights being lighter for each treatment) was probably due to the fact that the 1952 pigs were all farrowed by first litter gilts while some sows were used in the 1951 farrowing. Observations of qualitative effects, such as vigor of the newborn pigs, thrift and livability during the suckling period, and condition and nursing ability of the sows, indicated that no depressing effect

was being exerted by the aureomycin supplementation. There appeared to be a higher rate of survival of runt pigs on the highest level of aureomycin supplementation. In all groups, many of the pigs ate substantial amounts of feed from the sows' self feeders during the latter stages of the lactation period.

The average weaning weights showed no statistically significant differences among the treatment averages nor between years on the basis of the combined data. Within-year analyses showed that the weights

TABLE 2. SUMMARY OF DATA FROM GROWING-FATTENING TESTS

	Treatment (mg. of Aureomycin per lb. of Diet					
	1951			1952		
Item	10	50	100	10	50	100
Number of pigs	8	8	8	6	6	6
Av. initial weight, lb.	31.5	37.6	34.4	40.6	42.9	37.7
Av. final weight, lb.	214.3	222.4	217.7	218.9	221.0	217.4
Av. days on feed per pig	117	107	122	101	98	112
Av. daily gain, lb.	1.56	1.71	1.50	1.77	1.82	1.60
Feed per 100 lb. gain, lb	342	356	317	354	356	338

of the highest level group were significantly heavier in 1951 and lighter in 1952. The decrease in weaning weight in 1952 was believed to be the result of environmental conditions (a relatively high incidence of atrophic rhinitis in that group) rather than to treatment effect. A comparison between the 3 aureomycin-supplemented groups and a group receiving a similar diet without aureomycin showed a significant increase in average weaning weight for the supplemented groups (Davey and Stevenson, 1953).

The results from the growing-fattening studies based on the combined data of the two years are summarized in table 2. The average daily gains for the combined data show that the 50.0 mg. level supported the fastest gains (1.71 and 1.82 lb. per day) by a significant ($P<.05$) amount.

The figures for efficiency of gain show that in each year the pigs on the highest level made the most economical gains, although no statistical significance was found among groups. Some factor caused a limitation of feed intake for the high level group which in turn may have resulted in more effective use of the feed. A similar effect was

found by Ellis and Zeller (1934) who reported that limiting feed intake caused decreased rate of gain but increased efficiency of gain.

From general observations made during the growing-fattening phases of the study, it seemed that the pigs on the 50.0 mg. level had a certain bloom that was not found in the other groups. However, all groups appeared to be in good health throughout that portion of the experiment, with no indication of undesirable effects from the aureomycin supplementation.

Summary and Conclusion

Growth and reproduction studies were conducted on successive generations of swine fed levels of 10.0, 50.0, and 100.0 mg. of aureomycin per lb. of feed. The feeding of aureomycin at high levels produced no adverse effects in numbers of pigs born per litter, birth weights, or weaning weights. Evidence is presented which shows that a significant difference between treatments in average daily gain was obtained for the period from weaning to an average weight of 220 lb., the 50.0 mg. level supporting the most rapid gains in each generation.

Apparently neither the feeding of aureomycin continuously during the growth and reproduction periods nor the use of high levels of aureomycin had a deleterious effect on growth or reproduction.

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THE USE OF ARSANILIC ACID IN THE PRODUCTION OF MARKET PIGS [1]

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THE use of arsenic (Fowler's solution) as a tonic dates back to the eighteenth century. Roberts and Dawson (1935) have implied that the use of Fowler's solution in fitting show animals was a common practice for many years. In a rather extensive study with rabbits they found that this solution of potassium arsenite was in general detrimental to the health and fertility of rabbits and retarded growth in the young animals. This form of arsenic is much more toxic than the arsonic acids.

Interest in arsonic acids as feed additives for the improvement of growth in animals had its inception with the report of Morehouse and Mayfield (1946). These workers reported a marked growth stimulation in chickens and turkeys fed 3-nitro-4-hydroxyphenyl arsonic acid in the drinking water. Subsequent studies by Morehouse (1949) and by Bird *et al.* (1949), and others, showed that similar effects could be obtained by the direct addition of the arsonic acid to the diet.

Carpenter (1951) studied the value of 3-nitro-4-hydroxyphenyl arsonic acid for pigs and additional data have been reported from the Illinois Station, Becker *et al.* (1951). In a preliminary study at this Station (Anderson *et al.*, 1951) the addition of 0.005 percent or 0.01 percent of sodium arsanilate to a mixed ration for pigs increased the rate of gain approximately 11 percent.

The studies reported herein were initiated in 1952 to obtain information on the value of arsanilic acid as a "growth stimulant" for pigs, and particularly to measure the arsenic retention in the tissues of pigs fed this compound.

Experimental Procedure

This study included two experiments. The pigs used were purebreds, from the Station herd. They were farrowed indoors and were kept on

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concrete floors from birth and throughout the post weaning experimental period. They were self-fed, self-watered and provided with outside paved lots. The feeding plan was different in each experiment.

Experiment 1

Four lots of ten Poland China pigs each were used to compare three different levels of arsanilic acid fed in a mixed ration. The pigs were an especially thrifty appearing group with an average initial weight of

TABLE 1. COMPOSITION OF MIXTURES FED IN EXPERIMENT 1

Weight Range of Pigs	From Start to 125 lb. Average Weight	From 125 lb. to Slaughter
Est. Crude Protein, %	18	15
Ingredients	lb.	lb.
Yellow corn (ground No. 2)	66.7	77.0
Soybean oil meal (solv. proc.—44%)	15.3	10.0
Tankage (dry rendered—60%)	6.7	4.0
Linseed oil meal (solv. proc.—36%)	3.3	3.0
Alfalfa meal (dehydrated—17%)	6.7	5.0
Steamed bone meal (special)	0.7	0.5
Salt (iodized)	0.7	0.5
Vitamin B_{12} [1]	+	+

[1] B_{12} concentrate (Abbott) added at a rate calculated to supply 10 mg. of B_{12} per ton.

approximately 37 pounds. The treatments were as follows: Lot 1, Basal only (controls); Lot 2, Basal plus 30 gm. arsanilic acid per ton; Lot 3, Basal plus 60 gm. arsanilic acid per ton and Lot 4, Basal plus 90 gm. arsanilic acid per ton.

The feed mixtures used are given in table 1.

Most of the pigs were removed for slaughter at weights of 200–210 lb., on an individual basis. For reasons which will be discussed some were slaughtered at lighter weights. The pigs in the control group (Lot 1) were slaughtered the day they were taken off feed. In the other lots, some pigs from each lot were fed the experimental diets up to the time of slaughter; a few were placed on the control diet for 2 days, a few for 4 days and a few for 7 days before slaughter.

After slaughter and chilling, the carcasses were weighed, measured and processed. Samples of liver, kidney, muscle, fat and skin were taken from each carcass and quick frozen for later arsenic determinations.

Experiment 2

There were 8 Chester Whites and 2 Poland Chinas fed in each of 4 lots. The pigs were thrifty in appearance and weighed an average of 34.5 lb. at the start. The experiment was designed to compare 3 predetermined levels of protein with a self-selected (by the pigs) level, when all rations contain arsanilic acid. The mixed rations fed to lots 1, 2 and 3 are given in table 2.

TABLE 2. COMPOSITION OF MIXTURES FED IN EXPERIMENT 2

Weight Range of Pigs	Weaning to 125 lb.			From 125 lb. to Slaughter		
Lot number	1	2	3	1	2	3
Crude protein (planned) %	18	16	14	15	13	11
Crude protein (by analysis), %	18.7	16.5	13.0	14.6	12.5	10.2
Ingredients, lb.						
Yellow corn (ground No. 2)	68.7	74.4	79.6	78.6	83.6	88.5
Soybean oil meal (solv. proc.—44%)	15.3	12.0	8.7	9.2	6.0	2.8
Tankage (dry rendered—60%)	6.7	5.0	3.8	4.0	2.6	1.2
Linseed oil meal (solv. proc.—36%)	3.3	2.6	1.9	2.0	1.3	0.6
Alfalfa meal (dehydrated—17%)	5.0	5.0	5.0	5.0	5.0	5.0
Steamed bone meal (special)	0.5	0.5	0.5	0.7	1.0	1.4
Salt (iodized)	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin B_{12}[1]	+	+	+	+	+	+
Calcium pantothenate [1]	—	—	—	+	+	+
Arsanilic acid (crystalline)	60 gm. per ton for all mixtures					

[1] B_{12} concentrate (Abbott) added at a rate calculated to supply 10 mg. of B_{12} per ton. Calcium pantothenate (Merck) added at a rate of 2 grams per ton.

The pigs in lot 4 were allowed to select their own protein level. Ground corn was self-fed in one feeder and the following supplement in another feeder: 46 percent soybean oil meal, 20 percent tankage, 10 percent linseed oil meal, 20 percent dehydrated alfalfa meal, 2 percent steamed bone meal and 2 percent iodized salt. The supplement was fortified with 40 mg. vitamin B_{12} and 240 gm. of arsanilic acid per ton. After the pigs reached an average weight of 125 lb., 8 gm. of calcium pantothenate were added per ton of supplement, in addition.

The slaughtering and carcass evaluation procedures were the same as in Experiment 1, except that one animal in each lot was not slaughtered.

Results and Discussion

Feed Lot Data

Experiment 1. The first two weeks all lots of pigs gained less than one lb. per pig daily. The second two weeks the controls gained at a rate of 1.24 lb. per pig daily and the arsanilic acid-fed pigs gained

from 1.4 to 1.5 lb. per pig daily. The latter lots maintained a slight advantage in weight up to 125 lb., although there was some fluctuation between the treatment groups within the various weight periods. The feed lot data for 9 weeks, and statistical evaluation of the growth rate for the first 4 weeks of the experiment are given in table 3.

TABLE 3. THE INFLUENCE OF DIFFERENT LEVELS OF ARSANILIC ACID ON GROWTH RATE AND FEED EFFICIENCY OF PIGS FROM WEANING TO 125 LB. AVERAGE WEIGHT (10 PIGS PER LOT)

Lot No.	Arsanilic Acid/Ton	Av. Daily Feed	Av. Daily Gain	Feed/100 lb. Gain
	gm.	lb.	lb.	lb.
1	none	4.3	1.34	318
2	30	4.5	1.40	322
3	60	4.4	1.41	316
4	90	4.5	1.43	319

Analysis of Covariance (Growth Data for First 4 Weeks of Experiment)

Source of Variation	D.F.	Sum of Squares and Products			Error of Estimate		
		x^2	xy	y^2	Sum of Squares	D.F.	Mean Squares
Total	39	1667.1	2632.2	4813	657	38	
Between rations	3	2.5	8.0	126			
Within lots (error)	36	1664.6	2624.2	4687	550	35	15.7
For test of significance of adjusted lot means.					107	3	35.7

x=weight at start
y=weight after 4 weeks feeding

F=2.27
F=2.88 at P=.05

It appeared that arsanilic acid was most effective during the early part of the experiment. Hence, an analysis of covariance was made as indicated in table 3, and also at the 6th and 9th week of the experiment. The F value was 2.27 at 4 weeks ($F=2.87$ at $P=.05$) and this value diminished to 0.685 at 9 weeks. Thus, a difference in growth which approached significance after 4 weeks of feeding disappeared as the pigs grew heavier. There were no differences in feed efficiency between the treatment groups. All rations produced efficient gains.

Soon after the pigs reached the 125 lb. weight, lame pigs were noted in all lots. As the feeding period progressed the degree of lameness in some animals became severe. Examination of the feet of the affected pigs revealed deep fissures in the foot pads and cracked hooves. The pigs were carefully inspected by several veterinarians for evidence of vesicular exanthema and the findings were negative. Since the condition appeared in the control lot as well as in the lots of pigs fed arsanilic acid, it is clear that the feeding of the arsonic acid was not a cause of the ailment. The concrete floors were kept washed and no bedding was

used. Examination of the floors revealed a somewhat sharp and abrasive surface. However, pigs kept in these pens previously, under similar conditions of management, did not develop this condition. It is possible that this particular strain of breeding had peculiarly sensitive feet.

Because the pigs were affected in varying degrees and in varying numbers in the different lots the feed lot data from 125 lb. to slaughter weight have little meaning. The pigs were kept on the test rations in order to obtain carcass data and arsenic storage data. One pig died in lot 2 from a generalized septicemia arising in the area of an umbilical hernia. One pig died in lot 4 from an undetermined cause. Both deaths occurred in the latter part of the feeding period. There was no evidence of toxicity, due to the feeding of arsanilic acid, in any of the pigs.

Experiment 2. The growth and feed efficiency data are given in table 4, along with a statistical evaluation of the growth data. The pigs were fed to a slaughter weight range of 200–215 lb. Hence, the length of time each pig was on feed varied within lots as well as between lots. Therefore the statistical analysis reported includes only the data from the start and up to the point where the first pig was removed for slaughter.

The pigs fed the highest protein level (18–15 percent) consumed slightly more feed than any of the other lots and gained at a slightly faster rate than the pigs in lots 2 or 3 fed the mixed rations containing lower levels of protein.

The free-choice fed pigs (lot 4) consumed 3.8 lb. of corn in the first period and 5.4 lb. of corn in the second period, for each pound of supplement eaten. This consumption resulted in a protein level of 14.8 percent and 13.5 percent for the two feeding periods, respectively. The daily gain of these pigs was slightly higher than that of the pigs fed the 16–13 percent or the 14–11 percent protein mixed rations. The free-choice fed pigs consumed slightly less feed per lb. of gain than any of the other lots. The differences between the lots, for the entire feeding period, in rate of gain and efficiency of gain are small. In view of the protein content of the rations consumed by the free-choice fed pigs (lot 4) the differences between the lots in rate and efficiency of gain cannot be attributed to the protein levels fed. The analysis of covariance confirms this deduction, for the growth data. The F value was 0.744 ($F=2.87$ at $P=.05$). Additional analyses were made for the period from weaning to 125 lb. average weight ($F=1.358$) and for the period from 125 lb. to slaughter weight ($F=0.167$). None of the differences was significant at the 5 percent level.

Carcass Measurements

Experiment 1. Four pigs were slaughtered at weights of 153, 156, 170 and 171 lb., for reasons previously mentioned. The rest (34 pigs)

TABLE 4. GROWTH, AND FEED REQUIREMENTS OF PIGS FED DIFFERENT LEVELS OF PROTEIN PLUS ARSANILIC ACID.[1] (10[2] PIGS PER LOT)

Lot No.	Crude Protein in Ration		Av. Daily Feed	Av. Daily Gain	Feed/100 lb. Gain
	Planned	Analyzed			
	%	%	lb.	lb.	lb.
Early Growth—From Weaning 3 to 125 lb.					
1	18	18.7	4.5	1.42	313
2	16	16.5	4.2	1.37	304
3	14	13.0	4.2	1.35	308
4	F.C.[4]	14.8	4.3	1.47	294
Late Growth and Fattening—From 125 to 200 lb.					
1	15	14.6	7.3	1.81	401
2	13	12.5	6.6	1.67	393
3	11	10.2	6.7	1.67	401
4	F.C.[4]	13.5	6.4	1.71	373
Entire Feeding Period—From Weaning to 200 lb.					
1			5.6	1.59	355
2			5.1	1.50	344
3			5.2	1.48	353
4			5.2	1.57	330

Analysis of Covariance (Growth Data from Weaning to Final Weight)

Source of Variation	D.F.	Sum of Squares and Products			Error of Estimate		
		x^2	xy	y^2	Sum of Squares	D.F.	Mean Squares
Total	39	2533.8	5.37	1.25	1.235	38	
Between rations	3	1.7	0.13	0.07			
Within lots (error)	36	2532.1	5.50	1.17	1.161	35	0.0332
For test of significance of adjusted lot means					0.074	3	0.0247

x=weight at start
y=daily rate of gain

F=0.744
F=2.87 at P=.05

[1] 60 gm. arsanilic acid per ton of total ration for lots 1, 2 and 3. 240 gm. arsanilic acid per ton of supplement for lot 4.

[2] One pig died due to circulatory failure, in lot 2, during the "early growth" period. The data are calculated on a pig day basis.

[3] Initial weight was 34.5 lb.

[4] F.C.=free-choice fed; the protein level was calculated from analyses obtained for the corn and supplement fed.

were slaughtered at live weights of 190 to 216 lb. (average, 204 lb.). The carcass data for the latter are given in table 5.

Experiment 2. Nine pigs were slaughtered from each lot. One pig weighed 187 lb. and one weighed 222 lb., but most of them were in the range of 198 to 210 lb. The average for all lots was 207 lb. A summary of the carcass data is given in table 6.

TABLE 5. SUMMARY OF CARCASS MEASUREMENTS FROM HOGS FED DIFFERENT LEVELS OF ARSANILIC ACID—EXPERIMENT 1

Item	No. Pigs	Carcass Weight	Dressing Percent	Length of Carcass	Backfat Thickness	Carcass Yield (5 pr. Cuts)[1]
		lb.		cm.	cm.	%
		Lot 1—Basal Ration Only				
Mean	10	146.9	71.96	69.54	3.67	64.27
St. dev.[2]		4.19	1.01	2.34	0.55	1.29
		Lot 2—Basal+30 gm. Arsanilic Acid per Ton				
Mean	6	151.9	72.90	70.83	3.92	63.80
St. dev.		3.80	1.76	0.53	0.22	1.88
		Lot 3—Basal+60 gm. Arsanilic Acid per Ton				
Mean	10	145.0	72.36	69.68	3.65	64.56
St. dev.		3.05	1.44	1.89	.53	2.09
		Lot 4—Basal+90 gm. Arsanilic Acid per Ton				
Mean	8	148.8	73.04	69.95	3.70	63.86
St. dev.		2.90	1.74	0.07	.38	1.60

[1] Primal Cuts: including ham, loin, picnic, Boston butt and belly.
[2] Standard deviation.

There were no significant differences between rations, in either experiment, in dressing percent, length of carcass, backfat thickness or yield of the five primal cuts.

Arsenic Retention and Excretion

Fifty gm. samples of liver, kidney, muscle, fat and skin from each carcass were obtained at slaughter and quick frozen for later analysis.

TABLE 6. SUMMARY OF CARCASS MEASUREMENTS FROM HOGS FED ARSANILIC ACID WITH FOUR DIFFERENT LEVELS OF PROTEIN—EXPERIMENT 2

Item	No. Pigs	Carcass Weight	Dressing Percent	Length of Carcass	Backfat Thickness	Carcass Yield (5 pr. Cuts)[1]
		lb.		cm.	cm.	%
		Lot 1—18–15% Protein, Plus 60 gm. Arsanilic Acid				
Mean	9	152.6	72.68	71.72	4.44	59.89
St. dev.[2]		4.46	2.22	1.53	.48	1.61
		Lot 2—16–13% Protein, Plus 60 gm. Arsanilic Acid				
Mean	9	150.9	73.06	72.30	4.23	60.69
St. dev.		6.38	1.77	1.19	.57	4.13
		Lot 3—14–11% Protein, Plus 60 gm. Arsanilic Acid				
Mean	9	149.8	73.32	71.06	4.20	60.11
St. dev.		5.43	1.47	2.16	.59	2.86
		Lot 4—Self-Selected Protein Level, Plus 240 gm. Arsanilic Acid in Supplement				
Mean	9	151.8	72.81	71.22	4.19	60.67
St. dev.		5.11	2.02	1.50	.54	1.95

[1] Primal Cuts: includes ham, loin, picnic, Boston butt and belly.
[2] Standard deviation.

Assays for arsenic (As_2O_3) were made by the method of Cassil and Wichman (1939).

Experiment 1. A summary of the arsenic assays is given in table 7. These data show that the pigs fed arsanilic acid retained arsenic in their tissues in relation to the amount fed. Retention in the liver was approximately twice that in the kidneys. Arsenic retention in the muscle, fat or skin was rather low. The highest individual values ob-

TABLE 7. SUMMARY OF ARSENIC (As_2O_3) CONTENT OF TISSUES FROM SWINE FED ARSANILIC ACID (VALUES GIVEN ARE IN MICROGRAMS As_2O_3 PER GM. OF FRESH TISSUE)

Lot No.	Supplement	No. of Pigs	Days [1]	Liver	Kidney	Muscle	Fat	Skin
1	None	10	0	0.30	0.12	0.08	0.22	0.12
2	30 gm/ton	2	0	2.74	1.20	0.16	0.16	0.25
		3	2	1.48	0.42	0.16	0.30	0.12
		2	4	0.67	0.13	0.05	0.11	0.14
		2	7	0.37	0.19	0.06	0.26	0.26
3	60 gm/ton	3	0	3.98	2.04	0.26	0.05	0.43
		3	2	2.33	0.85	0.14	0.23	0.22
		2	4	0.87	0.28	0.14	0.13	0.11
		2	7	0.92	0.23	0.08	0.17	0.22
4	90 gm/ton	3	0	3.34	2.19	0.27	0.21	0.17
		2	2	3.40	0.74	0.26	0.17	0.14
		2	4	2.64	0.45	0.36	0.26	0.22
		2	7	1.38	0.19	0.14	0.20	0.11

[1] Days elapsed after withdrawal of arsanilic acid from the ration before the pigs were slaughtered.

tained were 0.37, 0.45 and 0.68 mcg. per gm. for muscle, fat and skin, respectively. For the same tissues from pigs not fed arsanilic acid the highest values found were 0.12, 0.52, and 0.36.

When arsanilic acid was removed from the diet for 2, 4 or 7 days prior to slaughter the arsenic content of the tissues was greatly reduced. It will be noted that the arsenic content of the liver of pigs slaughtered 4 days after arsenic withdrawal from the ration was approximately one-fourth as great as the values obtained from tissues of pigs slaughtered without arsenic withdrawal (pigs fed 30 or 60 gm. of arsanilic acid per ton of feed). The liver stores of the pigs fed the 90 gm. level were not reduced quite as rapidly.

Experiment 2. A summary of the arsenic assays is given in Table 8. The arsenic content of the fat and skin was not determined in this experiment because these tissues were shown to be very low in retained arsenic in Experiment 1 (see table 7) .

These results are similar to those obtained in the previous experiment. It is believed that the control lot in Experiment 1 may also serve as a control here, because the basal feed mixtures used were very similar in both experiments. As in the previous experiment, much more

arsenic was found in the liver and kidney, per gm. of tissue, than in the muscle. The pigs in lot 4, which were fed 240 gm. of arsanilic acid per ton of supplement retained somewhat less arsenic than the pigs

TABLE 8. SUMMARY OF ARSENIC (As_2O_3) CONTENT OF TISSUES FROM SWINE FED ARSANILIC ACID (VALUES ARE GIVEN IN MG. As_2O_3 PER GM. OF FRESH TISSUE)

No. Pigs	Days [1]	Liver	Kidney	Muscle
	Lot 1—18—15% Protein plus 60 gm. of Arsanilic Acid per Ton			
3	0	3.71	2.25	0.22
2	2	1.41	0.40	0.15
2	4	1.16	0.29	0.12
2	7	0.61	0.24	0.14
	Lot 2—16—13% Protein plus 60 gm. of Arsanilic Acid per Ton			
3	0	2.83	2.47	0.25
2	2	1.41	0.50	0.14
2	4	0.88	0.35	0.11
2	7	0.51	0.11	0.05
	Lot 3—14—11% Protein plus 60 gm. of Arsanilic Acid per Ton			
3	0	3.12	2.42	0.20
2	2	2.21	0.67	0.11
2	4	0.95	0.37	0.15
2	7	0.66	0.28	0.11
	Lot 4—Free-Choice Protein Level plus 240 gm. Arsanilic Acid per Ton of Supplement			
3	0	2.72	1.11	0.25
2	2	0.83	0.28	0.11
2	4	0.65	0.31	0.12
2	7	0.26	0.18	0.08

[1] Days elapsed after withdrawal of arsenic from the ration prior to slaughter.

fed the 60 gm. level in the total ration. This would be expected on the basis of the proportion of supplement in the total feed intake of these pigs. This experiment confirms a general conclusion of the first, namely, that when arsanilic acid is withdrawn from the ration for a few days prior to slaughter, the amount of arsenic remaining in the tissues is greatly reduced.

The relative arsenic excretion rates from the liver, kidney and muscle are shown in figure 1. In this figure, 100 percent represents the values obtained from pigs slaughtered without prior withdrawal of arsanilic acid from the ration.

This graph shows clearly that the excretion rate is greatest for the kidney and liver and slowest for the muscle. This would be expected on the basis of the very low retention of arsenic in the muscle, and on the basis of tissue function.

Frost (1953) has reported data on arsenic retention in tissues of chicks fed different levels of arsanilic acid. In general, his findings with chicks were similar to those of these experiments. However, the maximum retention in chick livers was less than that found in the livers of the pigs. Frost has reviewed the pharmacology and metabolism of arsenic compounds and also the literature on arsenic content of soils and foods. His review shows that many sea foods contain as much, or much more,

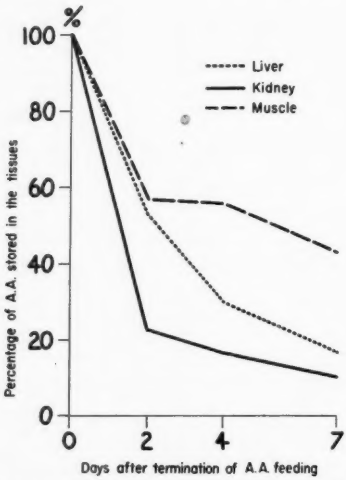

Figure 1. Relative rate of arsenic excretion.

arsenic than the livers of the pigs fed the 90 gm. level of arsanilic acid in experiment 1. The Food and Drug Administration limit for arsenic (spray residue) on fruits is 3.5 ppm.

In view of the results obtained in Experiment 1, the question may well be raised as to the advisability of feeding an arsonic acid to pigs. It should be kept in mind that the pigs used in that experiment were animals that most animal husbandmen would have classified at the start as being in excellent health and thrift. They were fed in a relatively sanitary environment. There has been no evidence of hemorrhagic enteritis in the Station herd, from which these pigs came.

Swine kept under a less favorable environment may react quite differently to the arsonic acids. Boley *et al.* (1951), Carpenter and Larson (1952) and Patrias (1952) have reported very favorable results from the use of arsonic acids in herds in which swine dysentery was a

problem. Furthermore, a response to sodium arsanilate was obtained in this herd in 1951 (Anderson *et al.*), a year in which enteric disorders were more prevalent in the herd.

Summary

In one experiment 40 pigs were fed in 4 lots to study the value of 3 different levels of arsanilic acid when added to a mixed ration. From the start of the experiment to an average weight per pig of 125 lb. the pigs fed arsanilic acid gained at an average rate approximately 5 percent greater than the controls. This difference was not significant ($P>.05$). There was no difference in feed efficiency due to the feeding of the arsonic acid. Growth and feed efficiency data beyond 125 lb. were confused by a problem not associated with the treatments.

In a second experiment 40 pigs were fed in 4 lots. Three of these were fed different predetermined levels of protein in mixed rations which contained 60 gm. arsanilic acid per ton of feed. The fourth lot was allowed to select its own protein level; they were fed ground corn and a mixed supplement free-choice. The supplement was composed of the same ingredients as were used to balance the corn in the mixed rations fed to the other 3 lots. For the free-choice fed pigs 240 gm. of arsanilic acid was added per ton of supplement. The differences between the lots in rate of gain and feed efficiency could not be related to the protein level of the rations fed, and differences in the liveweight gains were not significant at the 5 percent level.

There were no significant differences in carcass quality which could be attributed to the feeding of arsanilic acid (Experiment 1) or to the protein level of the ration (Experiment 2).

The pigs fed arsanilic acid retained small amounts of arsenic in the tissues and the amount retained was related to the level fed. Arsenic in the liver was approximately 1.5 to 2.0 times as great as the storage in the kidneys. The amount retained in the muscle, fat or skin was low at all levels of arsanilic acid fed. Removal of arsanilic acid from the ration prior to slaughter resulted in rapid excretion of arsenic from the liver and kidney. The rate of excretion from the muscle was less rapid. There was no evidence of toxicity in any of the pigs, in either experiment, due to the feeding of arsanilic acid.

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THE INFLUENCE OF ARSANILIC ACID ON PROTEIN REQUIREMENTS OF GROWING PIGS [1]

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THE protein "requirements" of pigs have been studied by various procedures over a period of many years. The recommendations coming from these studies have been somewhat variable. This is more understandable now, particularly in view of the newer knowledge relating to amino acid and vitamin requirements of the pig and the amino acid and vitamin content of commonly used feed stuffs.

Extensive feeding trials were conducted by the senior author several years ago to determine the protein requirements of pigs from weaning to market weight (Hanson, 1943–46). These studies showed that a 20–24 percent protein ration produced more rapid and efficient gains than a free-choice ration of lower protein content, when fed to pigs from weaning to 70 or 75 lb. From a weight of 65 to 125 lb., and 18-percent ration produced more rapid gains than a 15-percent ration, and from 125 to 200 lb. a 15-percent ration produced more rapid gains than a 13-percent ration. In these studies a mixture composed of equal parts of sardine meal, tankage, soybean oil meal and linseed oil meal was used to supplement corn and alfalfa meal. These data, in general, supported the findings of Keith and Miller (1940), who fed a ration which contained no fish meal. They were quite different than the recommendations of Crampton and Ashton (1942). The Canadian recommendations were based on a barley ration rather than corn and specified 15 to 18 percent protein from 30 to 100 lb. and 14 to 16 percent protein from 100 to 200 lb.

Following the discovery of vitamin B_{12}, Cunha *et al.* (1950) reported that the use of an "APF" supplement apparently reduced the protein requirement of the pig. These workers suggested that recommended protein levels might need revision. Catron *et al.* (1952) did reevaluate protein requirements with a corn-soybean oil meal type of

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ration and demonstrated clearly that the previously recommended allowances were higher than necessary. They also reported a protein "sparing-like" effect when aureomycin was fed.

In a previous experiment at this Station (Hanson *et al.*, 1955) there were no significant differences in rate or efficiency of gain when pigs were fed rations of low (14–11 percent) or high (18–15 percent) protein content. These rations contained 60 gm. of arsanilic acid per ton of feed. The experiment reported herein was designed specifically to determine whether the presence of arsanilic acid in the diet affects the protein requirement of the pig.

Experimental Procedure

The experiment consisted of 2 replicates with 8 lots of 5 pigs each per replicate. One replicate was started about a week earlier than the other, though both were fed during the fall and winter of 1952–53. The pigs used were purebred Durocs, Chester Whites and Poland Chinas from the Station herd. They were farrowed in the barn but were moved to a brome grass pasture at approximately 1 week of age. They were weaned at approximately 8 weeks of age and started on the experimental rations the same day. The pigs were sorted into groups of 5 on the basis of litter and weight and alloted to the various treatments by chance. In each replicate all but one of the pigs were barrows. The pigs were housed in a barn with outside paved runways. They were self-fed and an automatic water supply was provided. The experimental plan is given in table 1.

The feed mixtures fed to lots 1, 2, 3, 5, 6 and 7 are given in table 2.

Lots 4 and 8 were allowed to select their own protein levels. This was done by self-feeding ground corn in one feeder and the following supplement mixture in another feeder: 41 percent soybean oil meal, 20 percent tankage, 10 percent linseed oil meal, 25 percent dehydrated alfalfa meal, 2 percent steamed bone meal and 2 percent trace mineralized salt. This supplement mixture was fortified with 20 mcg. vitamin B_{12}, 120 mcg. folic acid, 4 mg. riboflavin, 8 mg. pantothenic acid, 18 mg. niacin, 20 mg. choline and 3600 U.S.P. units vitamin D per pound. This fortification rate was made on the assumption that the pigs would consume enough supplement to equal one-fourth of their total feed consumption. It should be noted (table 2) that in the mixed rations fed, the alfalfa meal was held constant and a constant ratio between soybean oil meal, tankage and linseed oil meal was maintained. The ratios between the latter three ingredients were approximately those of the supplement fed to lots 4 and 8.

TABLE 1. EXPERIMENTAL PLAN

Lot No.	Weaning to 125 lb.		From 125 to 200 lb.	
	Protein Level	Arsanilic Acid	Protein Level	Arsanilic Acid
	%	g/ton	%	g/ton
1	18	60	15	60
2	16	60	13	60
3	14	60	11	60
4	Free-choice	240[1]	Free-choice	240[1]
5	18	None	15	None
6	16	None	13	None
7	14	None	11	None
8	Free-choice	None	Free-choice	None

[1] Per ton of supplement.

Results and Discussion

The results of the two trials were combined and are summarized in table 3. In the first trial the pigs fed the 18 or the 16-percent-protein mixture gained more rapidly the first 2 weeks than the pigs fed the 14 percent mixture. After 4 weeks on feed the pigs on the low-protein mixture began to catch up to those on the higher protein levels and at the 125 lb. weight the differences between the protein levels were very small. In the second trial the growth curve was similar to the one obtained in the first trial, for the pigs not fed arsanilic acid. Those fed arsanilic acid gained at a similar rate in every weight period up to 125 lb., irrespective of protein level. The pigs fed free choice gained more rapidly from the start than the pigs fed mixed rations.

TABLE 2. COMPOSITION OF BASAL MIXTURES FED

Feeding Period	Weaning to 125 lb.			From 125 to 200 lb.		
Crude protein (estimated) %	18	16	14	15	13	11
Av. crude protein (by analysis) % [1]	18.6	16.0	14.7	16.0	13.8	11.0
Ingredients, lb.	%	%	%	%	%	%
Yellow corn (ground No. 2)	70.4	75.0	80.2	78.6	83.6	88.5
Soybean oil meal (44%)	14.5	11.5	8.2	9.2	6.0	2.8
Tankage (dry rendered—60%)	6.2	5.0	3.6	4.0	2.6	1.2
Linseed oil meal (36%)	3.1	2.5	1.8	2.0	1.3	0.6
Alfalfa meal (dehydrated—17%)	5.0	5.0	5.0	5.0	5.0	5.0
Steamed bone meal	0.3	0.5	0.7	0.7	1.0	1.4
Salt (trace mineralized) [2]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin supplement [3]	+	+	+	+	+	+

[1] Corrected to 12% moisture.

[2] Guaranteed to contain 0.250% Mn; 0.160% Fe; 0.033% Cu; 0.010% Co; 0.007% I_2 and 0.005% Zn.

[3] Vitamin supplement mixture provided the following additions to each pound of ration: 1 mg. riboflavin, 2 mg. pantothenic acid, 4.5 mg. niacin, 5 mg. choline, 30 micrograms folic acid, 5 micrograms B_{12} and 900 U.S.P. units vitamin D.

In each trial the pigs were weighed 4 times after the experiment started before they reached an average weight of 125 lb. This allowed 32 comparisons of average daily gains of the pigs fed arsanilic acid with

TABLE 3. THE INFLUENCE OF ARSANILIC ACID ON PROTEIN REQUIREMENTS OF GROWING PIGS (2 TRIALS WITH 10 PIGS PER TREATMENT) WINTER 1952–53

Lot No	Treatments: Protein Level	Treatments: Arsanilic Acid	Av. Daily Feed	Av. Daily Gain	Feed/100 lb. Gain
	Early Growth—Weaning[1] to 125 lb.				
	%	g/ton	lb.	lb.	lb.
1	18	60	5.1	1.66	310
5	18	0	5.0	1.59	318
2	16	60	4.8	1.61	297
6	16	0	4.9	1.58	308
3	14	60	4.8	1.60	301
7	14	0	4.5	1.56[2]	322
4	F.C. (14.4)[3]	240	5.3	1.72	308
8	F.C. (15.2)	0	5.2	1.62	314
	Late Growth and Fattening—125 to 200 lb.				
1	15	60	8.6	2.09	416
5	15	0	9.2	2.13	432
2	13	60	8.7	2.17	400
6	13	0	8.8	2.14	414
3	11	60	8.7	2.18	400
7	11	0	8.5	2.19[2]	412
4	F.C. (12.8)[3]	240	8.6	2.11	408
8	F.C. (12.2)	0	8.6	2.05	420
	Entire Feeding Period—Weaning to 200 lb.				
1	18–15	60	6.5	1.82	356
5	18–15	0	6.6	1.80	371
2	16–13	60	6.2	1.82	344
6	16–13	0	6.4	1.80	356
3	14–11	60	6.3	1.82	346
7	14–11	0	6.0	1.81[2]	364
4	14.4–12.8	240	6.6	1.86	351
8	15.2–12.2	0	6.5	1.79	362

[1] Initial weight in Trial 1 was 33 lb.; in Trial 2, 31 lb.

[2] One pig died and one failed to grow. The latter was removed in the early part of the experiment. The data are for 8 pigs.

[3] F.C.=free-choice; the protein level was calculated from analyses obtained for the corn and supplement fed.

those not fed the drug. (8 lots $\times$ 2 trials $\times$ 4 weights $=$ 64 weights; ½ fed arsanilic acid and ½ controls). In 30 of the 32 comparisons the pigs fed arsanilic acid were slightly ahead. In view of these consistent

but small differences, an analysis of covariance (Snedecor, 1946) was made. A summary of this study is given in table 4.

This analysis was made on the 78 pigs that were left at the 125 lb. weight. This study showed that the small differences between the gains of the pigs fed arsanilic acid and those not fed the drug were not significant at the 5 percent level. The analysis also showed that there were no significant differences between the ration treatments due to protein level. Additional covariance analyses were made of the live weight gains

TABLE 4. COVARIANCE ANALYSIS OF GROWTH DATA FROM WEANING TO 125 LB.

Source of Variation	D.F.	Sum of Squares and Products			Errors of Estimate		
		x^2	xy	y^2	Sum of Squares	D.F.	Mean Squares
Total	77	2870.187	6564.125	24455.9			
Protein levels	3	0.137	0.675	89.2			
Trials	1	90.312	114.750	145.8			
Arsanilic acid	1	0.112	−2.925	76.0			
All interactions	10	1.626	8.825	496.8			
Within sub class error	62	2778.000	6442.800	23648.0	8705.7	61	142.7
Pooled error	72	2779.626	6451.625	24144.8	9170.4	71	129.1
For testing significance of adjusted means of protein levels					86.9	3	28.9
For testing significance of adjusted means of trials					96.5	1	96.5
For testing significance of adjusted means of arsanilic acid					90.2	1	90.2

x=initial body weight
y=final weight

during the 125 to 200 lb. period and for the feeding period as a whole. These analyses revealed no significant differences due to arsanilic acid or to protein level.

The feed required per unit of gain was slightly in favor of the ration containing arsanilic acid at each protein level and during each feeding period. This was a consistent result in each trial. When the data are combined to give 2 lots, with and without arsanilic acid, the difference is approximately 4 percent in favor of arsanilic acid. Under the conditions of this experiment, however, arsanilic acid did not appear to have a direct "protein sparing" effect. There was no evidence of toxicity in any of the pigs fed arsanilic acid at any time during the course of the experiment.

Observations made during the experiment indicate that a mixed ration, with 14 percent protein of the type used, does not promote quite as rapid early gains as slightly higher protein levels, especially if the pigs weigh less than 35–40 lb. However, the data show that the pigs fed the low-protein mixtures did catch up later, and hence the practical significance of the somewhat slower start is of no real importance. These

data support the findings of Catron *et al.* (1952) that the high protein levels formerly recommended can be reduced, when the ration is otherwise well balanced. A recent report by Wallace *et al.* (1954) also supports the Iowa data, except that the Florida workers found that "there was no clear evidence that aureomycin enhanced protein utilization." On the other hand Burnside *et al.* (1954) report a protein sparing effect of aureomycin. The Iowa rations contained no alfalfa whereas the Florida and Wisconsin rations contained alfalfa. All were corn-soybean meal type rations.

The differences between the results obtained in this experiment and those of Hanson (1943–46) are perhaps due to the use of vitamin supplements in the present experiment. Calculations of the amino acid content of the low-protein mixtures fed in the present experiment indicate no serious deficiencies. On the basis of published values for amino acid content of feedstuffs the rations fed in the Nebraska experiments should have been fully equal in amino acid balance to the rations used in this experiment.

The free-choice-fed pigs in this experiment balanced their own rations in a fairly satisfactory manner. The ratio of corn to supplement eaten was as follows: From weaning to 125 pounds—Trial 1: lot 4, 4.3 to 1; lot 8, 3 to 1. Trial 2: lot 4, 4.3 to 1; lot 8, 4.1 to 1. From 125 to 200 pounds—Trial 1: lot 4, 6 to 1; lot 8, 6.5 to 1. Trial 2: lot 4, 6.4 to 1; lot 8, 9 to 1.

Summary

Six lots of 5 pigs each were fed in each of 2 trials to compare 3 mixed rations containing different levels of protein fed with and without arsanilic acid fortification. Two additonal lots of 5 pigs each, in each trial, were allowed to balance their own rations. One lot of each "protein-level" pair was fed arsanilic acid in the supplement.

The free-choice-fed pigs and those fed the mixed rations with 18 percent protein gained slightly faster during the period immediately following weaning. However, the pigs fed the lower-protein levels caught up later and reached market weight at essentially the same time as those fed the higher-protein mixed rations or those fed by the free-choice method.

The addition of 60 gm. of arsanilic acid per ton of mixed feed or of 240 gm. per ton of supplement (fed free-choice with ground yellow corn) produced small increases in rate of gain, at all levels of protein fed, from weaning to approximately 125 lb. live weight. These differ-

ences were not significant at the 5 percent level. The feeding of arsanilic acid produced a consistent increase of approximately 4 percent in feed efficiency.

Arsanilic acid did not appear to have a "protein-sparing" effect under the conditions of this experiment. There was no evidence of toxicity in any of the pigs fed arsanilic acid.

From the practical standpoint, the 14–11 percent protein mixed rations were as satisfactory as the mixed rations which were higher in protein.

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SOURCES OF UNIDENTIFIED FACTORS FOR THE PIG

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IN the past nutritionists have added various materials to poultry and swine diets which were believed to be complete in necessary nutrients known at the time, to test whether these added materials contained unidentified growth factors. This approach has led to the recognition and identification of several nutrients, particularly vitamins, which are important in feeding practices today. Since the discovery of vitamin B_{12} and its incorporation into synthetic diets in crystalline form, many sources of unidentified growth factors have been suggested in poultry nutrition. However, few studies have been conducted to ascertain the existence of unidentified growth factors for the pig.

The objective of the experiments reported here was to test several materials as sources of unidentified growth factor(s) for the pig fed a supposedly complete diet. In addition, studies were made to characterize the growth promoting or inhibiting agents encountered.

Experimental Procedure

A summary of the general experimental procedure is given in table 1. All experiments were of 8 weeks duration. In experiments 1, 2, 3 and 4, 32 weanling pigs were allotted at random to the 8 treatments from litter outcome groups composed of 8 or 4 pigs. In Experiment 5, 30 weanling pigs were allotted at random to the 6 treatments from litter outcome groups of 6 or 3 pigs. Because insufficient sets of littermate pigs were available for all replicates within an experiment, it was necessary to use 2 sets of littermate pigs for some replicates and thus some confounding resulted. In each experiment the pigs of each litter were randomly assigned to treatments and to cages or pens and feeding

[1] This paper is part of a thesis submitted by the senior author to the Graduate College, University of Illinois in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Animal Science. Present address: The Crete Mills, Crete, Nebraska.

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[3] Urbana, Illinois.

TABLE 1. GENERAL EXPERIMENTAL PROCEDURE

Exp. No.	Experimental Design	No. of Pigs	Diet of Dams	Type of Diet Used in Growth Tests	Method of Feeding	Ingredients Tested	% of Diet
1	2^3 factorial with 4 replications	32	Purified	Fortified corn-starch isolated soybean protein	Individually fed *ad libitum* on screens	Grass juice concentrate	3.0
						Dried whey-product with whey fermentation solubles	4.0
						Dried brewers' yeast	10.0
2	2^3 factorial with 4 replications	32	Purified	Fortified corn-starch isolated soybean protein	Individually hand fed full feed on concrete	Dehydrated alfalfa meal	10.0
						Menhaden fish solubles	3.0
						Streptomycin residue	2.0
3	2^3 factorial with 4 replications	32	Purified	Fortified corn-starch isolated soybean protein	Individually fed *ad libitum* on screens	Menhaden fish solubles	3.0
						Grass juice concentrate	3.0
						Dried whey-product with whey fermentation solubles	5.0
4	2^3 factorial with 4 replications	32	Practical diet on pasture	Fortified corn-soybean oil meal	Group-fed *ad libitum* on concrete	Menhaden fish solubles	3.0
						Grass juice concentrate	3.0
						Dried whey-product with whey fermentation solubles	5.0
5	2^3 factorial with 5 replications	30	Practical diet on pasture	Fortified corn-soybean oil meal	Group-fed *ad libitum* on concrete	Dehydrated alfalfa meal	10.0
						Streptomycin residue	1.8
						Woodflock	3.2

TABLE 2. COMPOSITION OF THE PURIFIED DIET

Ingredients	Percent
Corn starch	60.3
Drackett protein 220	19.7
Dextrose	10.0
Woodflock	3.0
Corn oil	2.0
Vitamin A and D oil (3000A, 600D)	0.5
DL-methionine	0.5
Mineral mixture No. 2 [1]	4.0
Vitamin mixture No. 46 [2]	+
Antibiotic mixture [3]	+
Total	100.0

[1] The percentage composition of mineral mixture No. 2 was as follows: $CaHPO_4$ 65.0, iodized NaCl 16.0, K_2CO_3 14.0, $MgCO_3$ 3.43, $FeSO_4 \cdot 7H_2O$ 1.00, $MnSO_4 \cdot H_2O$ 0.30, $CoCl_2 \cdot 6H_2O$ 0.10, $CuSO_4$ 0.10, NaF 0.02, $ZnCO_3$ 0.04, and KI 0.01.

[2] Vitamins added per pound of diet: (mg.) thiamine HCl 1.5, riboflavin 3.0, calcium pantothenate 15.0, niacin 20.0, folic acid 0.5, pyridoxine HCl 1.5, vitamin B_{12} 0.015, biotin 0.03, p-aminobenzoic acid 10.0, inositol 90.0, alpha-tocopherol acetate 5.0, 2-methyl-1,4-naphthoquinone 0.9, and choline chloride 600.0.

[3] A mixture of equal parts oxytetracycline HCl, chlortetracycline HCl, procaine penicillin, and streptomycin to supply 16 mg. of antibiotic per pound of diet. Added in experiment 2 only.

crates, and in Experiments 4 and 5 treatments were randomly assigned to pens.

The composition of the control diets for these experiments is given in tables 2 and 3. All diets were equalized for crude protein at 16 percent. The diets were equalized for crude fiber in Experiment 2, but it was not considered necessary in the other experiments.

The compositions of the condensed menhaden fish solubles and the dehydrated alfalfa meal were given by Gard (1954). The preparation

TABLE 3. COMPOSITION OF THE PRACTICAL DIET

Ingredients	Percent
Ground yellow corn	74.4
Solvent soybean meal	22.4
Steamed bone meal	1.0
Mineral mixture No. 3 [1]	2.0
Vitamin A and D concentrate (5000A–750D)	0.2
Vitamin mixture No. 37 [2]	+
Total	100.0

[1] The percentage composition of mineral mixture No. 3 was as follows: ground limestone 40.0, steamed bone meal 31.2, iodized NaCl 25.0, $MgCO_3$ 2.0, $FeSO_4 \cdot 7H_2O$ 1.0, $MnSO_4 \cdot H_2O$ 0.56, $CoCl_2 \cdot 6H_2O$ 0.10, $CuSO_4$ 0.10, and $ZnCO_3$ 0.04.

[2] Vitamins added, mg. per pound of diet: Thiamine HCl 0.5, riboflavin 1.4, niacin 5.0, calcium pantothenate 4.5, pyridoxine HCl 0.6, folic acid 0.35, inositol 90.0, p-aminobenzoic acid 10.0, biotin 0.03, vitamin B_{12} 0.005, and choline chloride 454.

of the grass juice concentrate was described by Kohler and Graham (1951). This material contained 50.0 percent solids, 5.0 percent protein and 12.5 percent ash. The dried whey-product with whey fermentation solubles[4] contained 51.7 percent lactose, 17.7 percent protein, 14.1 percent minerals, 1.5 percent fat, 4.0 percent acid (as lactic), 4.0 percent moisture and no fiber. The streptomycin residue sample tested in Experiment 2 contained 20.9 percent protein; whereas the sample tested in Experiment 5 contained 19.8 percent protein, 49.5 percent ash, 4.4 percent fat, 0.7 percent calcium, 3.4 percent phosphorus and approximately 0.38 mg. of vitamin B_{12} per lb. This material is a spray dried sample of residue from the production of streptomycin. The dried brewers' yeast had a crude protein content of 47.4 percent.

Final weights were analyzed by covariance in each experiment to adjust for differences in initial weight. Feed consumption data in Experiments 1, 2 and 3 and feed required per lb. of gain in Experiment 2 were analyzed by covariance to adjust for differences in initial weight. In Experiments 1, 2 and 3 final weights were analyzed by covariance to adjust for differences in feed consumption in addition to differences in initial weight. Two pigs had to be removed in Experiment 3 and the missing values were accounted for by dummy variates.

Results

Experiment 1

The pigs averaged 27.8 lb. at the start, consumed 3.26 lb. of feed per head daily, and required 2.81 lb. of feed per pound of gain. The analysis of adjusted final weights is given in table 4.

The interaction of grass juice concentrate and dried whey was significant at the 5 percent level. Grass juice concentrate and dried whey each increased the average daily gain significantly (P less than 0.01) over that of pigs receiving neither, but their effects were not additive and pigs receiving both did not gain significantly better than those receiving either one (table 5). These data suggest that grass juice concentrate and dried whey are contributing the same factor to the diet and that this factor is required in small amounts.

The analysis of feed consumption data indicated that pigs receiving grass juice concentrate consumed significantly (P less than 0.05) more feed than pigs receiving no grass juice concentrate. When final weights were also adjusted for differences in feed consumption the grass juice concentrate-dried whey interaction was not significant, though nearly reaching the 5 percent level.

[4] This ingredient is usually referred to as dried whey in the remainder of this paper.

TABLE 4. ANALYSIS OF ADJUSTED FINAL WEIGHTS. EXPERIMENT 1

Source of Variation	d/f	Mean Square
Litters	6	274**
Treatment	(7)	
Grass juice concentrate (G)	1	338
Dried whey (W)	1	17
Dried brewers' yeast (Y)	1	22
G×W	1	221*
G×Y	1	1
W×Y	1	49
G×W×Y	1	15
Error	17	41

* Significant at 5 percent level.
** Significant at 1 percent level.

Experiment 2

The pigs had an average initial weight of 26.5 lb. and consumed an average of 2.73 lb. of feed daily. The analysis of feed efficiency data indicated that pigs receiving streptomycin residue required significantly (P less than 0.05) more feed per lb. of gain than pigs that received no streptomycin residue (2.26 vs. 2.19 lb. of feed).

The analysis of adjusted final weights is given in table 6, and table 7 shows average daily gains (adjusted for differences in initial weight) for each of the eight treatments. The 3-factor interaction was significant at the one percent level, showing that each factor affects growth. The adjusted average daily gains indicate that dehydrated alfalfa meal and streptomycin residue both depressed gains. Their effects were not additive since pigs receiving both made about the same gain as those receiving either. Furthermore, fish solubles significantly (P less than 0.05) increased the gains but had no effect in the presence of either or both of the other factors.

Analysis of feed consumption data also showed a significant interaction of all 3 factors. Alfalfa meal and streptomycin residue each depressed feed consumption significantly below the controls, but not in combination. Fish solubles had little effect alone or in the presence

TABLE 5. TWO-WAY TABLE OF ADJUSTED AVERAGE DAILY GAINS IN LB. EXPERIMENT 1

	No Dried Whey	Dried Whey	Av.
No grass juice concentrate	0.98	1.19	1.08
Grass juice concentrate	1.22	1.25	1.24
Average	1.10	1.22	1.16

TABLE 6. ANALYSIS OF ADJUSTED FINAL WEIGHTS. EXPERIMENT 2

Source of Variation	d/f	Mean Square
Litters	4	120*
Treatment	(7)	
Dehydrated alfalfa meal (A)	1	168
Menhaden fish solubles (F)	1	2
Streptomycin residue (S)	1	487
A×F	1	113
A×S	1	282
F×S	1	14
A×F×S	1	244**
Error	19	25

* Significant at 5 percent level.
** Significant at 1 percent level.

of alfalfa meal but increased feed consumption in the presence of streptomycin residue and decreased it in the presence of both streptomycin residue and alfalfa meal. When final weights were also adjusted for differences in feed intake, there was still a significant (P less than 0.05) interaction between alfalfa meal and streptomycin residue, each reducing final weight but not lowering it further in combination.

Experiment 3

The pigs had an average initial weight of 23.5 lb., consumed 2.81 lb. of feed per head daily, and required 2.60 lb. of feed per lb. of gain. Adjusted treatment and error mean squares for final weights and feed consumption are shown in table 8.

Pigs receiving grass juice concentrate made significantly (P less than 0.01) faster gains than the pigs receiving no grass juice concentrate (1.16 vs. 1.00 lb. daily) and consumed significantly (P less than 0.01) more feed per head daily than the pigs receiving no grass juice concentrate (2.97 vs. 2.65 lb. daily). When a multi-variate test was made, significance dropped to the 5 percent level, indicating feed consumption and final weight are probably different measures of the same thing, as

TABLE 7. THREE-WAY TABLE OF ADJUSTED AVERAGE DAILY GAINS IN LB. EXPERIMENT 2

	No Alfalfa		Alfalfa	
	No Fish Solubles	Fish Solubles	No Fish Solubles	Fish Solubles
No streptomycin residue	1.32	1.46	1.25	1.15
Streptomycin residue	1.15	1.13	1.19	1.14

TABLE 8. MEAN SQUARES OF FINAL WEIGHT (W) AND FEED CONSUMPTION (F), AND THEIR CROSS-PRODUCTS, ADJUSTED FOR INITIAL WEIGHT. EXPERIMENT 3

Source of Variation	d/f	W^2	WF	F^2
Litters	5	117.8*	209.3	549.1*
Treatment	(7)			
Fish solubles (S)	1	58.1	153.3	404.6
Grass juice concentrate (G)	1	425.6**	833.3	1631.3**
Dried whey (D)	1	12.1	4.1	1.4
S×G	1	50.9	82.1	132.5
S×D	1	193.8*	412.5	878.3*
G×D	1	50.1	74.7	11.4
S×G×D	1	6.3	7.2	8.2
Error	16	40.9	73.8	168.0

* Significant at 5 percent level.
** Significant at 1 percent level.

was suggested by their very high error correlation, 0.89. This was further indicated when final weights were adjusted for differences in feed consumption and vice versa, no significant difference being found.

Fish solubles × dried whey interaction was significant (P less than 0.05) for gains and feed consumption. A two-way table for adjusted average daily gains and feed consumption involving fish solubles and dried whey is shown in table 9.

Dried whey caused slower growth rate and lower feed consumption compared to controls but the depressions were restored by fish solubles. No significant difference was observed in the multivariate test. When final weights were also adjusted for differences in feed consumption or vice versa no significant difference was found. From these analyses it appears that final weight and feed consumption were influenced by the same character.

TABLE 9. TWO-WAY TABLE OF ADJUSTED AVERAGE DAILY GAINS AND AVERAGE DAILY FEED CONSUMPTION PER PIG IN LB. EXPERIMENT 3

	No Fish Solubles		Fish Solubles		Average	
	Gain	Feed	Gain	Feed	Gain	Feed
No dried whey	1.08	2.83	1.05	2.78	1.06	2.81
Dried whey	1.02	2.64	1.17	2.99	1.09	2.81
Average	1.05	2.73	1.11	2.88	1.08	2.81

Experiment 4

The pigs had an average initial weight of 25.3 lb., consumed an average of 2.82 lb. of feed daily and required 2.42 lb. of feed per lb. of gain. Analysis of adjusted final weights is given in table 10.

TABLE 10. ANALYSIS OF ADJUSTED FINAL WEIGHTS. EXPERIMENT 4

Source of Variation	d/f	Mean Square
Litters	5	112
Treatment	(7)	
Menhaden fish solubles (F)	1	211
Grass juice concentrate (G)	1	1159*
Dried whey (W)	1	1
F×G	1	23
F×W	1	11
G×W	1	8
F×G×W	1	22
Error	18	67

* Significant at 1 percent level.

The growth rate of the pigs receiving grass juice concentrate was significantly (P less than 0.01) faster than for the pigs receiving no grass juice concentrate (1.34 vs. 1.10 lb. daily). Although pigs receiving menhaden fish solubles did not show a significantly improved feed conversion or growth response, they required 0.08 lb. less feed per lb. of gain and gained at a somewhat faster rate than pigs receiving no menhaden fish solubles (1.21 vs. 1.12 lb. daily).

Experiment 5

The pigs on this experiment had an average initial weight of 33.0 lb. and consumed an average of 3.15 lb. of feed daily. Pigs fed streptomycin residue required 2.71 lb. of feed per lb. of gain. Pigs fed dehydrated alfalfa meal required 2.92 lb. of feed, pigs fed the crude-fiber equivalent of dehydrated alfalfa required 2.76 lb. of feed and pigs fed neither dehydrated alfalfa meal nor its crude-fiber equivalent required 2.59 lb. of feed per lb. of gain. Analysis of adjusted final weights is shown in table 11.

The two-factor interactions, crude fiber sources × litters in part A, and crude fiber sources × litters in part B were not statistically significant when tested against the three-factor interaction, crude fiber sources × litters × streptomycin residue (from part A only). Hence,

these three interactions were pooled and used as the error term to test the remaining sources of variation. Although no statistically significant difference was observed in this experiment, the effect of streptomycin residue had an F value of 4.76, whereas 4.84 is required for significance (P=0.05). The pigs fed streptomycin residue gained 1.26 lb. daily as compared to 1.03 lb. for those not fed streptomycin residue. This is contrary to the results obtained with the purified diet. Differences in the diet fed, in the streptomycin residue samples tested, or in the

TABLE 11. ANALYSIS OF ADJUSTED FINAL WEIGHTS. EXPERIMENT 5

Source of Variation	d/f	Mean Square
Parts [1] (P)	1	77
Streptomycin residue (S)	1	822
P×S	1	2
Crude fiber sources (C)	2	333
C×P	2	98
C×S	2	67
C×P×S	2	119
Litters (L) in part A	2	184
Litters (M) in part B within S	2	107
L×S	2	166
(C×M)+(C×L)+(C×L×S)	11	173

[1] The 3 sets of 6 littermate pigs were considered as part A; the 4 sets of 3 littermate pigs were considered as part B.

amount included in the diets of these two experiments may account for these discordant results.

When an amount of woodflock (3.2 percent) equivalent to the crude fiber content of 10 percent dehydrated alfalfa meal was added to the control diet, no depression in growth was observed. However, 10 percent dehydrated alfalfa meal depressed growth considerably, from 1.20 to 1.03 lb. gain per day. Apparently the crude fiber content in the dehydrated alfalfa meal was not responsible for this growth depression.

Discussion

The growth response of the weanling pig to several possible sources of unidentified factors has been investigated. When Wahlstrom (1951) included 3 percent grass juice concentrate or 3 percent condensed fish solubles in an alpha-protein "synthetic milk" baby-pig diet the growth rate was increased 8 and 15 percent, respectively. In our studies reported here menhaden fish solubles failed to exert a clear-cut favorable effect on the growth of weanling pigs. The growth response to grass juice concentrate reported by Wahlstrom is somewhat similar to the

response observed here when growth is not adjusted for differences in feed intake.

Grass juice concentrate significantly increased growth rate in each of the three experiments reported here in which it was included. Legg *et al.* (1950) and Cheng *et al.* (1952) observed that grasses and legumes showed estrogenic activity. Furthermore, Burroughs *et al.* (1954) observed that diethylstilbestrol in the diet of steers produced a marked improvement in growth and feed conversion. Hence, it was postulated that the pig's growth response to grass juice concentrate might be due to its estrogenic content. Therefore the grass juice concentrate was assayed for estrogenic activity by the rat uterine weight method, using one month old castrate female rats. A standard dose-response curve was established with estradiol benzoate to compare with the dose response of grass juice at various levels. The maximum uterine growth response produced by the grass juice concentrate indicated that 1 ml. contained no more than the equivalent of 0.016 mg. of estradiol benzoate. Thus, in experiments where 3 percent grass juice concentrate was included in the rations, the pigs receiving grass juice concentrate may have consumed the approximate equivalent of 0.7 mg. of estradiol benzoate daily. This level related to body weight agrees with the levels used in ruminant tests.

Dried whey influenced growth significantly in only two of the three experiments in which it was tested, depressing in one and stimulating in the other. Terrill and Krider (1950) and Briggs and Beeson (1952) did not obtain a growth response with 5 percent and 2 percent dried whey, respectively, when added in the presence of vitamin B_{12}. Vohs *et al.* (1951) found that 2 percent dried whey added to a ration fortified with vitamin B_{12} produced an increased growth rate.

The growth response to menhaden fish solubles in this series of tests agrees with the observations of Terrill *et al.* (1952) who reported that 3 percent menhaden fish solubles substituted for vitamin B_{12} and bacitracin did not alter the growth rate significantly. Anderson and Black (1952) obtained an 8 percent increase in average daily gains over the control pigs when 3 percent sardine condensed fish solubles was included in the ration. Even when vitamin B_{12} was included in the ration, Stephenson *et al.* (1951) concluded that fish solubles contributed an unidentified "vitamin-like" factor.

In the experiments in which 10 percent dehydrated alfalfa meal was tested a 14 percent growth depression resulted. These results are similar to those of Krider *et al.* (1947) who found that 10 percent dehydrated alfalfa meal depressed growth approximately 10 percent, but contrary

to the results of Krider and Terrill (1950) who observed that including 10 percent dehydrated alfalfa meal was more nearly optimum than 4 percent of this product, in a vitamin B_{12} deficient diet. Dinusson *et al.* (1953) indicated that little growth depression was observed when alfalfa meal was included at levels lower than 30 percent of the ration.

Similar growth tests with chicks have indicated that high levels of alfalfa meal depressed growth. In addition, Cooney *et al.* (1948) found that crude fiber was not responsible for the growth depressing effect of alfalfa meal when included in the chick ration above 5 percent. The evidence presented here also indicates that some factor other than fiber accounts for the growth depressing property of alfalfa meal. The recent studies of Peterson (1950) and Heywang and Bird (1954) suggest that saponin is the growth inhibiting principle. Potter and Kummerow (1954) demonstrated that both dehydrated alfalfa and soybean meal contained saponins which were shown to have chick growth depressing properties equivalent to that present when these feedstuffs formed 18 to 20 percent of the diet. Thus in the present series of tests dehydrated alfalfa meal and the main protein source probably contributed enough saponins to cause a marked growth depression compared with the control ration containing soybean oil meal but no dehydrated alfalfa meal.

Summary

A total of 158 weanling pigs was used in a series of five factorial experiments to test dehydrated alfalfa meal, menhaden fish solubles, grass juice concentrate, dried brewers' yeast, a dried whey-product with whey fermentation solubles, and a streptomycin residue, as possible sources of unidentified growth factors. The pigs were allotted at random to the treatments from litter outcome groups. Individual feed consumption data were collected in 3 of the experiments. Both purified and practical diets were used. All rations within an experiment were equalized for crude protein. The final weights for each experiment were analyzed according to the method of covariance to adjust for differences in initial weight. In the tests where individual feed consumption data were available final weights were also adjusted for differences in feed consumption.

According to the statistical analyses of adjusted final weights, grass juice concentrate produced a significant growth response. This growth response approached significance when final weights were also adjusted for differences in feed consumption. The significant interactions of grass juice concentrate with dried whey and of menhaden fish solubles with

dried whey were not significant when feed consumption was considered. These ingredients influenced the voluntary feed intake and, in addition, grass juice concentrate may be supplying an unidentified growth factor.

An assay of the grass juice concentrate for estrogenic activity indicated that 1 ml. contained no more than the equivalent of 0.016 mg. of estradiol benzoate.

Dehydrated alfalfa meal fed at the 10 percent level depressed growth. The crude fiber content of the dehydrated alfalfa meal was not the primary factor causing this growth depression because a crude fiber equivalent did not depress growth. A streptomycin residue depressed growth and feed efficiency when incorporated in a purified diet at the 3.0 percent level but promoted growth when included in a practical ration at the 1.8 percent level.

There was no clear-cut evidence that dried brewers' yeast, dried whey-product with whey fermentation solubles, or menhaden fish solubles contained unidentified growth factors for the pig.

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SOME EFFECTS OF ADDING SUPPLEMENTS OF LYSINE, METHIONINE AND TRYPTOPHAN TO PRACTICAL SWINE RATIONS [1]

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EXPERIMENTS designed to determine the individual essential amino acid requirements of weanling pigs have been reported. The requirement for lysine is based on the work of Mertz *et al.* (1949), Brinegar *et al.* (1950a, b), and Shelton *et al.* (1951a); the requirement for methionine on the work of Shelton *et al.* (1951b) and Curtin *et al.* (1952); and the requirement for tryptophan on the work of Beeson *et al.* (1948) and Shelton *et al.* (1951c). The requirements have been summarized by the National Research Council (1953) and by Almquist (1954). Comparison of these requirements with published analyses of conventional swine feeds indicates that many practical rations may be deficient in lysine, methionine or tryptophan.

Bloss *et al.* (1953), and Terrill *et al.* (1954) have shown that tryptophan improves a corn-meat and bone scraps ration for swine and preliminary reports of the value of adding lysine to rations of milo-soybean meal (Pond *et al.*, 1953), corn-soybean meal (Catron *et al.*, 1953) and a mixed ration (Pfander and Tribble, 1953) were made. Dyer *et al.* (1949) reported a growth response when a corn-soybean meal ration was supplemented with methionine.

Suberlich (1954) has suggested that antibiotics spare lysine in rat rations.

The experiments reported in this paper were conducted to determine the effect of supplementing practical swine rations with l-lysine HCl, dl-methionine and dl-tryptophan.

Experimental

Three experiments were conducted with Chester White pigs from weaning until they weighed about 100 lb. Two basal rations were used.

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[3] Columbia.

Ration A was composed of corn and a supplement of soybean meal 2, tankage 2 and wheat shorts 1. The ratio of corn and supplement was varied to obtain desired protein levels. Ration B was formulated from corn and soybean oil meal. The initial protein level was 18 percent in trial 1 and 16 percent in trials 2 and 3. When the pigs weighed approximately 75 lb. the level of protein was reduced to 16 percent in trial 1 and 14 percent in trials 2 and 3. Both rations were fortified with vitamins and minerals to reach the levels recommended by the

TABLE 1. COMPOSITION OF THE BASAL RATIONS

Component	Ration A			Ration B		
	Percent	Crude	Protein	Percent	Crude	Protein
	18	16	14	18	16	14
Ground yellow corn [1]	742.5	787.5	839.5	727.5	761.5	817.5
Soybean oil meal, 44% solvent [2]	100	80	58	250	211	155
Tankage, 60% [2]	100	80	58	..	..	..
Wheat gray shorts	50	40	29	..	..	..
Vitamins [3]	1	1	1	1	1	1
Choline chloride, 25% dry mix	0.5	0.5	0.5	0.5	0.5	0.5
Limestone	..	5.0	8.0	12.5	12.0	9.0
Salt	5	5.0	5.0	5.0	5.0	5.0
Bonemeal	..	..	..	2.5	8.0	11.0
Antibiotic [4]	+	+	+	+	+	+

[1] Amount of corn varied slightly, depending on the antibiotic used. Sufficient corn was added to make 1000 lb. of ration.

[2] Blended from at least two sources.

[3] Each lb. contained 7 gm. nicotinic acid, 7 gm. pantothenic acid, 1 gm. riboflavin, 4 mg. vitamin B_{12}, 976,500 I.U. vitamin A and 173,600 I.U. vitamin D.

[4] One lb. TM 10 in trial 1, 6 lb. Aureofac in trial 2, and 3 lb. of Aureofac 2A in trial 3.

National Research Council (1950). The ingredients in the basal rations are shown in table 1. Rations were self fed in trials 1 and 2 and were hand fed in trial 3.

Litters of pigs were weaned at 56 days of age and allocated to the experimental treatments according to the 6×6 or 7×7 Latin square design. When the pigs weighed about 100 lb. in trials 1 and 3 and 120 lb. in trial 2, they were removed from the experiment. All pigs were kept in a barn with concrete floored pens and had access to adjacent concrete floored lots.

Ration ingredients and mixed rations were analyzed for nitrogen by the method of Kjeldahl and for amino acids by microbiological methods.

Experiment 1

Six litters of 6 pigs were allocated to the following 6 lots: Lot 1, basal A; Lot 2, basal A+0.15 percent l-lysine; Lot 3, as 2+0.04 per-

cent dl-methionine; Lot 4, basal B; Lot 5, basal B+0.15 percent l-lysine; Lot 6, as 5+0.04 percent dl-methionine.

Experiment 2

Seven litters of 7 pigs were allocated to 7 lots: Lot 1, basal A; Lot 2, basal A+0.1 percent l-lysine; Lot 3, basal A+0.04 percent dl-methionine; Lot 4, as 2+0.04 percent dl-methionine; Lot 5, basal B; Lot 6, basal B+0.1 percent l-lysine; Lot 7, basal B+0.04 percent methionine.

Experiment 3

The treatments were as follows:

Lot 1	Basal A—antibiotic	
2	"	+lysine+methionine+tryptophan
3	"	
4	"	+lysine+tryptophan
5	"	+lysine
6	"	+lysine+methionine
7	"	+lysine—antibiotic
8	"	+tryptophan
9	"	+methionine
10	"	+tryptophan+methionine

The levels of supplement used were: 0.1 percent for l-lysine added as the hydrochloride, 0.04 percent for dl-methionine, and 0.04 percent for dl-tryptophan.

A 6×6 Latin square design was used to allot pigs to lots 2–7. Lots 1 and 8–10 contained 6 pigs similar to those in lots 2–7.

Results and Discussion

Growth Rate

The average daily gains of the pigs are summarized in tables 2–4. The lots receiving ration A supplemented with lysine always gained more than the lots receiving the basal ration but the difference was not significant in any one trial, because a pig in one or more litters grew faster on the basal ration than any of his litter mates did on the supplemented ration. In table 5 the average daily gains of all pigs receiving the supplemented rations are compared to the mean gain of all pigs of the same sex within a litter. The difference between the basal group and the lysine supplemented group was significant ($P<0.05$). The difference between the basal group and the group receiving lysine and methionine supplements was not significant.

The response of the pigs to lysine in trial 3 was nearly as great when the rations contained no antibiotic (Lot 1 vs. 7) as in the anti-

TABLE 2. THE EFFECT ON GROWTH RATE AND FEED EFFICIENCY OF SUPPLEMENTING PRACTICAL SWINE RATIONS WITH L-LYSINE AND DL-METHIONINE, (EXPERIMENT 1, SPRING 1953)

Lot number	1	2	3	4	5	6
Ration	Basal A	A+L[1]	A+L[1]+M[2]	Basal B	B+L[1]	B+L[1]+M[2]
No. of pigs	6	6	6	6	6	6
Av. initial wt., lb.	35.0	34.3	32.5	34.0	33.0	34.8
Av. final wt., lb.	98.3	102.5	96.8	99.3	95.7	101.3
Av. daily gain, lb.	1.29	1.39	1.29	1.34	1.24	1.36
Av. daily feed, lb.	3.78	3.90	3.53	3.93	3.58	4.07
Feed/cwt. gain, lb.	293	280	274	294	289	300

[1] 0.15 percent of l-lysine was added as the hydrochloride.
[2] 0.04 percent of dl-methionine was added.

Analysis of Variance of Average Daily Gains

Source	d/f	M.S.
Between treatment	5	16.9
Between litters	5	59.*
Between sex	1	25.
Between basal rations	1	15.
Error	23	10.35

* $P<.01$.

biotic-containing ration (Lot 3 vs. 5). There was no evidence that aureomycin spared lysine.

Pigs receiving a supplement of 0.04 percent dl-methionine in trials 2 and 3, 0.04 percent of dl-tryptophan, or 0.04 percent dl-tryptophan +

TABLE 3. THE EFFECT ON GROWTH RATE AND FEED EFFICIENCY OF SUPPLEMENTING PRACTICAL SWINE RATIONS WITH L-LYSINE AND DL-METHIONINE, (EXPERIMENT 2, FALL 1953)

Lot	1	2	3	4	5	6	7
Ration	Basal A	A+L[1]	A+M[2]	A+L[1]+M[2]	Basal B	B+L[1]	B+M[2]
No. of pigs	7	7	7	7	7	7	7
Av. initial wt., lb.	31.4	30.3	30.4	30.9	31.0	30.6	30.1
Av. final wt., lb.	113.7	120.2	126.4	119.0	119.3	120.9	115.0
Av. daily gain, lb.	1.33	1.44	1.56	1.42	1.43	1.46	1.34
Av. daily feed, lb.	4.58	4.63	4.80	4.36	4.55	4.32	4.15
Feed/cwt. gain, lb.	343	321	309	307	318	295	309

[1] 0.10 percent l-lysine was added as the hydrochloride.
[2] 0.04 percent dl-methionine was added.

Analysis of Variance of Average Daily Gains

Source	d/f	M.S.
Between treatments	6	362.2**
Between litters	6	691.5**
Between sex	1	147*
Between basal rations	1	36
Error	34	27.4

* $P<.05$.
** $P<.01$.

TABLE 4. THE EFFECT ON GROWTH RATE AND FEED EFFICIENCY OF SUPPLEMENTING PRACTICAL SWINE RATIONS WITH L-LYSINE, DL-METHIONINE AND DL-TRYPTOPHAN, (EXPERIMENT 3, SPRING 1954)

Lot. No.	1	2	3	4	5	6	7	8	9	10
Ration	−AB[4]	+L[1]M[2]T[3]	Basal	+L[1]T[3]	+L[1]	+L[1]M[2]	−AB[4]+L[1]	+T[3]	+M[2]	+M[2]+T[3]
No. of pigs	6	6	6	6	6	6	6	6	6	6
Av. initial wt., lb.	32.3	31.2	31.3	31.5	31.3	30.5	31.5	31.2	30.8	30.0
Av. final wt., lb.	95.7	100.5	95.9	103.8	107.5	104.3	98.7	102.0	102.8	96.0
Av. daily gain, lb.	1.11	1.22	1.13	1.27	1.34	1.30	1.18	1.25	1.27	1.16
Av. feed consumed, lb.	3.51	3.64	3.61	3.76	3.69	3.66	3.63	3.64	3.64	3.61
Feed/cwt. gain, lb.	316	300	319	296	275	283	306	291	287	312

[1] 0.10 percent l-lysine was added as the hydrochloride.
[2] 0.04 percent dl-methionine was added.
[3] 0.04 percent dl-tryptophan was added.
[4] —AB—Same as basal ration, but contained no antibiotic.

Analysis of Variance of Average Daily Gains Lots 2–7

Source	d/f	M.S.
Between treatments	5	114.2
Between litters	5	338.6*
Between sex	1	11
Error	24	100.5

* $P < .05$.

lysine or lysine + tryptophan + methionine in trial 3 grew at a faster rate than pigs on the basal lots.

One of the most unexpected results was the failure of the combination of lysine, methionine and tryptophan or of any two of these amino

TABLE 5. THE DIFFERENCE IN MEAN DAILY GAIN (FROM ALL PIGS OF THE SAME SEX WITHIN A LITTER) OF THE PIGS RECEIVING RATION A, RATION A PLUS L-LYSINE, AND RATION A PLUS LYSINE PLUS DL-METHIONINE, (Summary of Three Trials)

Litter	Difference from Mean Daily Gain of All Pigs of Same Sex Within a Litter		
	Basal A	+ Lysine	+ Lysine and Methionine
	lb.	lb.	lb.
1	+0.17	−.02	+.08
2	0	−.03	0
3	− .13	−.03	+.04
4	− .03	+.21	−.18
5	− .18	+.05	+.04
6	0	0	+.01
7	−.16	+.20	−.21
8	− .02	+.01	−.17
9	− .15	−.15	+.10
10	+ .05	+.10	−.18
11	− .13	+.25	−.03
12	− .13	−.20	−.10
13	+ .08	−.02	+.21
14	+ .18	0	−.08
15	0	−.06	−.07
16	− .45	+.15	+.04
17	− .15	+.25	−.06
18	− .13	+.14	+.21
19	− .20	+.06	+.20
Total	−1.38	+.86	−.15
Average	− .07	+.05	−.01

Students t, basal vs. lysine 2.3
" t, basal vs. lysine+methionine 1.38
" t, for P=0.05 2.101

acids in combination to produce a growth stimulation as great as that which was produced by lysine alone. The added amino acids may have been rapidly absorbed from the digestive tract, entered the metabolic pool and been catabolized and excreted before the bound amino acids of the ration were freed by digestive enzymes and entered the metabolic pool. Sheffner and Bergeim (1953) have shown that excess dietary amino acids increase the rate of l-amino acid oxidation by rat kidney,

and Schweigert (1947) and Suberlich *et al.* (1948) have shown that urinary excretion of free and bound amino acids is influenced by the ration.

In two trials, pigs did not grow faster when supplements of lysine, methionine, or lysine + methionine were added to the basal ration B. Dyer *et al.* (1950) obtained a growth stimulation when a 20 percent crude protein corn-soybean oil meal ration was supplemented with methionine but other investigators using lower levels of protein have not obtained growth responses from supplemental methionine (Ferrin, 1946; Catron, 1953). Corn protein contains about 3 percent methionine and 1.6 percent cystine (Flynn *et al.*, 1954) and soybean meal proteins contain about 1.7 percent methionine and 1.7 percent cystine. Therefore, as the percent of protein in a corn-soybean oil meal ration decreases the concentration of sulfur amino acids increases.

The failure of supplemental lysine to cause a growth response to a corn-soybean meal ration containing 14 or 16 percent protein was reported by Catron *et al.* (1953).

Feed Consumption

There were no significant differences in the daily feed consumption of the supplemented and basal rations, but pigs usually consumed more of ration A when it was supplemented with lysine or methionine and more of ration B when it was not supplemented. When both lysine and methionine were present the daily feed consumption of the lots was similar to that of the basal group.

Feed Efficiency

The addition of lysine to ration A and B increased feed efficiency over the basal lots ($N=5$, $P<0.05$).

The addition of methionine to basal A and B increased feed efficiency ($N=3$, $P<0.02$). The addition of lysine and methionine increased feed efficiency of ration A ($P<0.05$) but did not increase feed efficiency of ration B.

In trial 3 pigs receiving supplements of tryptophan, tryptophan + lysine, and tryptophan + lysine + methionine required 7 percent less feed per cwt. gain than the pigs in the basal lot.

The average increased feed efficiency obtained when the lysine supplemented rations were fed was sufficient to produce an additional 50 lb. of live hog per ton of feed, indicating that lysine supplementation of rations similar to those used in these trials would be economical if one could buy 2 lb. of lysine for less than the value of 50 lb. live hog.

Discussion

Since no information on the biological availability of the rations was obtained, it is difficult to explain the failure of lysine to stimulate the growth rate when added to ration B. On the basis of published compositions and amino acid assays of the feed used in these trials, basal ration A contained as much or more lysine than basal ration B. The lysine content, expressed as a percent of the protein, of basal ration A was 4.8, 4.6, 4.3 and of ration B was 4.7, 4.5 and 4.3 when they contained 18, 16 and 14 percent crude protein.

The amounts of amino acids present in supplemented ration A containing 16 percent crude protein, (0.83 lysine, 0.56 methionine + cystine and 0.16 percent tryptophan) are less than the recommended allowances of the National Research Council for pigs weighing between 25 and 70 lb. (1.0 lysine, 0.6 percent methionine and 0.2 percent tryptophan). However, if the amino acid requirements are expressed as a percent of the protein, the levels which were satisfactory in these studies more nearly approach those recommended by the National Research Council (lysine, 5.2 vs. 6.0; methionine, 3.5 vs. 3.6; and tryptophan, 1.0 vs. 1.2). It seems desirable to make recommendations in terms of percent of protein rather than the percent of ration. This system has been recommended by Almquist (1953) for poultry and by Cornell, *e.g.* Brinegar (1950) for swine.

Becker *et al.* (1954) have reported that a corn-soybean meal ration containing 14 percent of crude protein was satisfactory but a 16 percent corn-fish meal ration was required under their conditions. The corn-soy ration contained 0.63 percent lysine, 0.23 percent methionine (no measurement of cystine) and 0.13 percent tryptophan. This corresponds to 4.5, 1.65 and 0.9 percent of the protein. The fish meal ration contained greater concentrations of lysine and methionine but was though to be deficient in tryptophan.

Soybean meal has greater concentrations of glycine, phenylalanine + tyrosine, sulfur amino acids and arginine than shorts or tankage. Therefore, the addition of lysine to a soybean-corn ration should make a more balanced ration. Shelton *et al.* (1951) obtained a lower lysine requirement (4.2 percent of protein) on a ration containing 10 percent gelatin which is high in glycine, than was obtained by Brinegar *et al.* (1950) on rations that did not supply large amounts of glycine. The higher requirements obtained by Brinegar (1950) may have resulted from the use of unbalanced proteins, large levels of free amino acid supplements and from shortages of one or more nutrients in the ration.

Many of the published requirements are based on free amino acid supplementation of rations which are nearly free of the amino acid under test—for example, (Brinegar *et al.*, 1950 and Mertz *et al.*, 1949) used rations very low in lysine and supplemented them with free lysine. It is doubtful that the utilization of an amino acid mixture would be the same when a few of the amino acids are being supplied in pure form and others are joined in the protein molecules. Cunha *et al.*, (1949) have shown that the requirement for methionine is lower when vitamin B_{12} is present in the ration and the conversion of tryptophan to niacin is well known (Luecke, 1947, 1948, Powick, 1948). Salmon (1954) has suggested that a downward revision of amino acid requirements will be possible when rations well balanced in amino acids are formulated.

Comparison of the growth rate of the pigs on the present experiments during the same period of time as the amino acid requirements were determined by Brinegar and Mertz indicates that the pigs on these rations grew as fast or faster than those receiving high levels of lysine.

The fact that some pigs on the unsupplemented rations grew at faster rates than their litter mates receiving the supplemented rations indicates that the lysine requirement is variable. Before controlled nutritional experiments can produce additional refinements in requirement figures, the variability within litters must be reduced or the requirement will have to be stated as a range of values instead of an exact figure.

Summary

Three trials were conducted with pigs from weaning to about 100 lb. to determine the effect of supplementing practical rations of corn, soybean meal, tankage and wheat shorts containing 14, 16 and 18 percent protein with lysine, methionine and tryptophan. The addition of 0.10 percent of l-lysine increased growth rate and feed efficiency but did not greatly increase feed consumption. Supplements of methionine or tryptophan increased growth rate and feed efficiency but combinations of two or three amino acids did not give as great response as lysine alone.

Two trials were conducted to determine the effect of supplementing a corn and soybean meal ration with lysine and methionine. Neither of the supplements alone or in combination increased growth rate but they did improve feed efficiency slightly.

The requirements of the pig, expressed as a percent of the protein, appear to have been met by 5.0 percent l-lysine, 3.5 percent methionine + cystine and 1.0 percent tryptophan.

Discussion

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HISTIDINE—AN ESSENTIAL NUTRIENT FOR GROWTH OF PIGS

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HISTIDINE has been included among the indispensible amino acids for the growth of swine (Mertz *et al.*, 1952) although a search of the literature reveals no actual published data in this regard. Preliminary observations (Brinegar, 1951) suggested that growing pigs may require histidine, but the data were not conclusive. The present studies were undertaken in the hope of clearing up the problem of whether or not a dietary source of histidine is required for the growth of swine. Studies were made using amino acids and diammonium citrate as the only dietary nitrogen sources.

Experimental Procedure and Results

Experiment 1

A preliminary experiment involving 5 young Yorkshire pigs was conducted to test an experimental "milk-type" diet containing purified amino acids and diammonium citrate as the only nitrogen sources. Two pigs were fed what was considered to be a "complete" amino acid diet, while two more received the same diet minus histidine. The fifth pig received a stock diet containing casein as the protein source, serving as a positive control.

The amino acid diets appeared to be very unpalatable, and a rather severe diarrhea was encountered. Sucrose, which had been used as the source of carbohydrate in the diets, is believed to be largely responsible for this diarrhea, since it has recently been shown that young pigs cannot utilize sucrose well and that diarrhea is encountered when it is fed (Becker *et al.*, 1954a, 1954b). Due to the difficulties encountered, the results of this preliminary trial were inconclusive, but there was an indication that histidines may be required for growth of the young pig.

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Experiment 2

The amino acid diets used in the preliminary experiments were modified somewhat and a second experiment was conducted. The composition of the experimental diets used is given in table 1. Six littermate York-

TABLE 1. COMPOSITION OF AMINO ACID DIETS
(GM. per KG. of Liquid Diet)

Ingredients	Basal	Basal Plus Histidine
Amino acids	16.281	17.276
L-arginine HCl	1.206	1.206
Dl-isoleucine	2.144	2.144
L-leucine	1.675	1.675
L-lysine HCl	2.345	2.345
Dl-methionine	1.340	1.340
Dl-phenylalanine	2.010	2.010
Dl-threonine	2.412	2.412
Dl-valine	2.814	2.814
Dl-tryptophan	.335	.335
L-histidine HCl H_2O	....	.995
Monosodium glutamate	1.34	1.34
Liver powder	0.50[1]	0.50[1]
Diammonium citrate	15.08	13.748
Glucose (cerelose)	59.19	59.727
Lard	30.00	30.00
Tween[2]	0.30	0.30
Minerals[3]	11.71	11.71
Vitamins[4]	+	+
Aureomycin HCl[5]	+	+

[1] After 8th day of experiment, was replaced with 0.4 gm. of diammonium citrate and 0.1 gm. of glucose.
[2] Composed of Tween 80, 50%; Tween 60, 50%. Supplied by Atlas Powder Company, Wilmington, Delaware.
[3] Minerals of Sewell *et al.*, 1953.
[4] Vitamins of Eggert *et al.*, 1954; plus 0.01 mg. of biotin per kilogram of liquid diet.
[5] Contained 150 mg. per kg. of dry diet. Supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

shire pigs were removed from the sow at 6 days of age and individually fed a stock casein diet for a few days. As the pigs were small at birth, they were fed this stock diet for 8 days, at the end of which time (2 weeks of age) they ranged in weight from 2740 to 3065 gm., and were all eating and gaining satisfactorily. At this time the pigs were allotted into 2 groups according to weight and sex. Three of the pigs were then shifted to the basal diet while the other 3 were shifted to the diet containing histidine. The change from the stock diet to the amino acid

diets was made gradually, three days being used to make the complete change. Appetites remained good and gain in body weight continued normally for all pigs during this changeover period. Once the change was completed, however, the pigs receiving the basal diet exhibited a markedly slower growth rate, while those receiving the diet with histidine continued to increase in body weight at a fairly rapid rate. Each pig was weighed at 2-day intervals.

After 6 days on the experimental diets it was evident that while the growth rate of the pigs receiving the basal diet was markedly decreased, none of these three pigs was actually losing weight. Since the possibility existed that the small amount of histidine contained in the diet might be allowing this slow growth, the liver powder was removed from both diets entirely on the eighth day of the experiment.

The three control pigs receiving histidine were removed from the experiment after 18 days on the amino acid diet, since their growth response seemed to indicate that the diet was fairly satisfactory. Their growth was not, however, as good as would be expected on a stock diet in which the same amount of nitrogen would be furnished by casein or skimmilk. Afted 22 days on the histidine-free diet two of the pigs were very weak and thin, and their feed consumption had decreased considerably. Although they were not actually losing weight, they were gaining only very slowly (4.5 and 8.4 gm. per day). At this time these two pigs were changed to the diet containing the histidine for 14 days. The other pig (63) was still eating fairly well at this time, and was increasing in weight at a steady and somewhat more rapid rate than the other two pigs. This pig was continued on the histidine-free diet for another 6 days, making a total of 28 days on the histidine-free diet. At the end of this 28-day period her body weight was still increasing, but her appetite was becoming more erratic, and this pig was also changed to the diet containing histidine for a period of 14 additional days.

The immediate effect on each of the three pigs upon being changed to the diet containing histidine was a marked increase in appetite. Feed consumption nearly doubled in from 24 to 48 hours after the diet change was made. An immediate upswing was apparent in the growth curve for each pig, however since the first measure of weight gain was made 48 hours after the change in diet, by which time the feed consumption was nearly doubled, one cannot tell whether the increase in appetite preceded increased growth or vice versa.

About a week after the diet change was made, feed consumption generally became erratic, and growth responses were irregular. Whether this condition was due to the accumulative effect of some undetermined diet deficiency or whether perhaps it might be due to digestive disturbances caused by the rapid increase in feed consumption was not readily apparent. One pig (64) went almost completely off-feed after receiving the diet containing added histidine for 12 days. This pig lost weight very severely for the last two days of the experiment. She was restless the next day after being removed from the experiment. Convulsions set in that evening and death occurred during the night. No autopsy was performed. The pig which was carried the longest on the basal diet (63) quit eating two days after the experiment was terminated. Symptoms were generally similar to that observed for pig number 64, except that no convulsions were noted. Autopsy showed a hypertrophied heart with an area of degeneration at the apex. The remaining pig (60) which had received the basal diet was sacrificed at this time to see if any abnormalities might be found in the heart or other tissues. Internally this pig appeared normal and healthy in all respects, and histological examination of the tissues failed to show any abnormalities.

As to just what may have caused the heart lesions is difficult to say. It is possible that they could have been the direct result of the histidine deficiency, but the evidence for this is not convincing. It is not believed to be due to vitamin destruction in the diet, since the water soluble vitamins were kept as a premix with glucose in a refrigerator and added at the time of feeding. The fat soluble vitamins were added to the fat homogenate and kept under refrigeration until fed.

A summary of daily gains and feed efficiency for each pig is shown in table 2. Although the pigs receiving the histidine-free diet did increase in body weight somewhat, it is clearly evident that some histidine is needed for good growth of young pigs. An analysis of variance of the average daily gains showed that the pigs fed the basal diet plus histidine gained significantly faster (less than 0.01) than the pigs fed the basal diet. The fact that the pigs did not lose weight and that one actually gained slightly without histidine suggests that the quantitative requirement of the pig for histidine for growth may be low, and that perhaps there may be some slow synthesis of this amino acid either in the intestinal tract or the tissues of the young pig. Considerably more data are needed for any final conclusion on these points.

TABLE 2. GROWTH AND FEED EFFICIENCY OF PIGS RECEIVING AND NOT RECEIVING HISTIDINE

	With Histidine			No Histidine			With Histidine After Depletion		
Pig number	65	61	62	63	60	64	63	60	64
Days on trial	18	18	18	28	22	22	14	14	14
Starting weight, gm.	3300	3360	3095	3345	3130	3170	4140	3315	3270
Final weight, gm.	5075	5680	4910	4140	3315	3270	5680	4725	3900
Average daily gain, gm.	98.6	128.9	100.8	28.4	8.4	4.5	110.0	100.7	45.0[1]
Daily feed consumption, gm. (air-dry basis)	220.5	230.8	212.6	169.2	155.7	114.4	244.2	233.2	181.5
Air-dry feed per kg. of gain, kg.	2.24	1.79	2.11	5.96	18.54	25.42	2.22	2.32	4.03

[1] Daily gain for the first 12 days of the 14-day period was 98.3 gm. per day. Pig died 2 days after the experiment was finished—see discussion.

Summary

Young pigs receiving a semi-synthetic diet containing all ten of the amino acids known to be required for the growth of the weanling rat grew at a fairly rapid rate, although not as rapidly as would be expected on a diet in which the same amount of nitrogen is furnished by casein, while pigs receiving the same diet minus histidine grew much slower or failed to grow at all. When histidine was added to their diets, these pigs showed greatly increased appetites and resumed growth almost immediately. It is concluded that histidine is required for the normal growth of young pigs.

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EFFECTS OF THE ADDITION OF DEHYDRATED ALFALFA MEAL, FISH SOLUBLES AND A "VITAMIN B_{13}" CONCENTRATE TO A PURIFIED DIET FOR SOWS

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A SATISFACTORY purified diet for use in swine gestation and lactation studies had not been developed by research workers in the past. Ensminger *et al.* (1946), Russell *et al.* (1948), Hogan and Anderson (1948) and Anderson and Hogan (1950) used purified diets that were probably inadequate since all of the present known vitamins, especially vitamin B_{12}, were not included.

Fairbanks *et al.* (1945) with a practical diet and Ensminger *et al.* (1951) with a purified diet, demonstrated that alfalfa meal had a favorable effect upon gestation and lactation performance, although the control diets appear somewhat deficient in vitamin B_{12}. Heidebrecht *et al.* (1949) reported that the average number of pigs weaned increased slightly when alfalfa meal and fish solubles were added to a practical control diet. DePape *et al.* (1953) observed a significant adverse effect on litter weaning weights and number weaned when dehydrated alfalfa meal was substituted for sun-cured alfalfa meal in a practical ration adequate in vitamin B_{12}.

The objectives of the three experiments reported herein were (1) to develop a purified diet for comparing sources of unidentified factors and for use in studying nutrient requirements for gestation and lactation, and (2) to determine the effects of adding 10 percent dehydrated alfalfa meal to a purified gestation and lactation diet. In Experiment 2 the effect of adding 3 percent menhaden fish solubles or a "vitamin B_{13}" concentrate to the diet were studied as well as the effect of adding 10 percent dehydrated alfalfa meal.

[1] This material is based upon part of a thesis submitted by the senior author as partial fulfillment of the requirements for the Ph.D. degree at the University of Illinois. Present address: The Crete, Mills, Crete, Nebraska.

[2] The authors wish to acknowledge the aid of H. W. Norton in the statistical analysis of the data and W. W. Moore for examining the reproductive tracts.

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Experimental Procedure

Animals Used

Experiment 1. Four pairs of littermate gilts were divided into 2 lots according to weight and then randomized for treatment. The gilts had been raised in drylot except for being on pasture 3 weeks prior to the start of the experiment. Matings were started 6 days after the gilts were started on the diets.

Experiment 2. Five litter-outcome groups were allotted to the 4 treatments in a manner similar to that used in Experiment 1. The gilts were on the diets 5 weeks before matings were started.

Experiment 3. The sows from Experiment 2 were reallotted according to previous treatment. The sows on the control diet and those on the control diet plus "vitamin B_{13}" concentrate during Experiment 2 formed the new control lot. The sows that had received dehydrated alfalfa meal or menhaden fish solubles during Experiment 2 were pooled to form a new group to receive a supplement of 10 percent dehydrated alfalfa meal. At the conclusion of the experiment the sows were bred and slaughtered 3 to 9 days later. Reproductive tracts and internal organs were examined.

Criteria and Statistical Analysis

The main criteria of the effects of the diets during gestation were weight, number and strength of pigs at birth. The main criteria during lactation were percent of pigs weaned, feed required per pound total gain of sow and litter during gestation and lactation, and individual and litter weaning weights. The litter weaning weights were subjected to statistical analysis since they are a fairly representative indication of over-all gestation-lactation performance. The data from the sows fed the basal diet and from the sows fed the control diet plus 10 percent dehydrated alfalfa meal in all 3 experiments were analyzed by fitting constants since there were unequal treatment numbers within a given experiment.

Feeding and Management Practices

During gestation the animals were hand-fed in treatment groups with feed consumption per lot equalized on a per head basis. During lactation each sow and litter was full fed, either by self-feeding or hand-feeding. To prevent nutritional anemia, each sow's udder was swabbed periodically with an iron sulfate solution until the pigs were eating the

sow's feed or their creep feed. In Experiment 2, a creep feed the same as the dam's feed was available to the pigs. All three experiments were conducted on concrete.

About 5 days before farrowing the dams were washed and placed in individual pens that had been cleaned thoroughly and disinfected. In Experiment 2, the gilts were moved 1 to 2 days before farrowing to

TABLE 1. CONTROL DIETS FED DURING GESTATION AND LACTATION

Ingredients	Experiments 1 and 2	Experiment 3
	lb.	lb.
Corn starch	63.9	62.8
Alpha-protein	16.0	—
Drackett protein 220	—	17.2
Dextrose	10.0	10.0
Woodflock	3.0	3.0
Corn oil	2.0	2.0
Vitamin A and D oil (3000A, 600D)	0.5	0.5
DL-methionine	0.6	0.5
Mineral mixture No. 2 [1]	4.0	4.0
Vitamin mixture No. 46 [2]	+	+

[1] The percentage composition of mineral mixture no. 2 was as follows: 65.0 $CaHPO_4$, 16.0 iodized NaCl, 14.0 K_2CO_3, 3.43 $MgCO_3$, 1.00 $FeSO_4 \cdot XH_2O$, 0.30 $MnSO_4 \cdot H_2O$, 0.10 $CoCl_2 \cdot 6H_2O$, 0.10 $CuSO_4$, 0.02 NaF, 0.04 $ZnCO_3$ and 0.01 KI.

[2] Vitamins added in milligrams per pound of ration: 1.5 thiamine HCl, 3.0 riboflavin, 15.0 calcium pantothenate, 20.0 niacin, 0.5 folic acid, 1.5 pyridoxine HCl, 0.015 vitamin B_{12}, 0.03 biotin, 10.0 p-aminobenzoic acid, 90.0 inositol, 5.0 a-tocopherol acetate, 0.9 2-methyl-1, 4 naphthoquinone and 600 choline chloride.

farrowing stalls, if available. Two days after farrowing the dams and litters were moved to individual pens equipped with guard rails and infrared heat lamps. Wood shavings were used for bedding.

Diets Fed

The control diets fed are given in table 1. An alpha-protein was used as the protein source in Experiments 1 and 2, whereas Drackett protein 220 was used in Experiment 3. Since alpha-protein has a fairly high sulfite content (0.96 to 1.10 percent SO_2) the alpha-protein content was equalized in the diets of Experiments 1 and 2. The sulfite content was considered less critical with the Drackett protein 220, thus the diets of Experiment 3 were equalized for crude protein. All diets were made as nearly equal in crude fiber content as possible by varying the amounts of woodflock.

In Experiments 1 and 2 each gilt received a daily supplementation of 7.5 ml. of an alcohol solution containing 1.2 gm. of thiamine HCl

per liter; likewise, each week she received 9.25 gm. of a vitamin A and D oil containing 3000 I.U. of vitamin A and 600 I.C.U. of vitamin D per gram. After Experiment 2 had been in progress approximately 3 months it was deemed advisable to give each gilt a supplemental amount of alpha-tocopherol acetate in a water-alcohol solution (3.5 ml. per gilt daily of a solution containing 50 gm. of alpha tocopherol per liter of 20 percent alcohol). During lactation the above vitamin supplements were doubled. No daily or weekly vitamin supplementations were made in Experiment 3 since Drackett protein 220, a low sulfite material, was used as the protein source in the diet.

The 3 samples of dehydrated alfalfa meal tested in the 3 experiments contained a minimum of 17.0 percent protein, 1.5 percent ether extract, 39.0 percent nitrogen-free extract and a maximum of 27.0 percent fiber. The condensed menhaden fish solubles tested in Experiment 2 contained 49.5 percent solids, 36.7 percent protein, 4.8 percent ether extract and it had a pH value of 4.1. The "vitamin B_{13}" concentrate tested in Experiment 2 was extracted from corn distillers solubles by a modification of the method of Austin and Boruff (1949). The method used is as follows: Extraction of distillers solubles with chloroform, solvent removal and extraction of residue with methanol, solvent removal and extraction into water followed by concentration of the aqueous extract and extraction again with chloroform. The residue from the final chloroform extraction is dissolved in 70 percent alcohol for feeding experiments. One ml. of the material was equivalent to 3 lb. of corn distillers solubles.

Samples of all diets were taken at mixing. Proximate analysis was made on a composite sample of each control diet. Composite samples of other diets were checked for crude protein and crude fiber.

Results and Discussion

Gestation results for all three experiments are presented in table 2 and lactation results in table 3.

Control vs. Control Plus 10 Percent Dehydrated Alfalfa Meal

The effect of adding 10 percent dehydrated alfalfa meal to a purified gestation and lactation diet was observed in all three experiments.

In Experiment 1 the number of services per conception was the same for each treatment. In Experiment 2, one of the gilts on the dehydrated alfalfa meal treatment was bred at 2 heat periods before conception occurred.

TABLE 2. RESULTS DURING GESTATION

Items Compared	Experiment 1		Experiment 2				Experiment 3	
	Lot 1 Control	Lot 2 +10% Alfalfa	Lot 1 Control	Lot 2 +10% Alfalfa	Lot 3 +3% Fish Sol.	Lot 4 +"B_{12}" Conc.	Lot 1 Control	Lot 2 +10% Alfalfa
Number of sows started on test	4[1]	4	5	5	5	5[2]	9[3]	9[4]
Number of services per conception	1.0	1.0	1.0	1.2	1.0	1.2	1.4	1.0
Number of sows farrowed	2	4	5	5	5	4	5	7
Av. initial weight per sow, lb.	251	250	222	219	217	218	369	377
Av. daily gain to farrow, lb.	1.15	1.38	1.27	1.17	1.27	1.33	1.00	1.16
Av. daily feed, lb.	4.9	5.3	5.0	5.0	5.0	5.0	5.0	4.9
Av. no. pigs farrowed per litter	3.5	8.8	8.4	7.4	9.4	8.2	7.6	9.3
Av. birth weight per pig, lb.	2.97	2.73	2.86	2.96	2.69	3.19	3.02	2.92
Percentage of pigs farrowed:								
Strong	100	66	76	81	57	88	74	77
Medium	0	11	12	3	17	0	10	11
Weak	0	6	2	0	4	0	10	3
Alive but strength unknown	0	0	2	3	19	3	0	3
Dead	0	17	7	14	2	9	5	6

[1] One gilt died from the heat after 92 days of pregnancy. A second gilt was slaughtered after she failed to farrow and was observed to be cycling.

[2] One gilt was slaughtered after she came in heat regularly 4 times. A complete constriction of the cervix was observed.

[3] Four sows never farrowed. First sow died of heat stroke 57 days after being bred. The second sow returned in heat 79 days after being bred and was slaughtered. Upon examination a cystic right fimbria was observed. Third sow was slaughtered 3 days after she was due to farrow. Possible resorption took place. The fourth sow was observed in heat 110 days after being bred. An examination of the reproductive tract indicated she had been cycling normally.

[4] One sow broke a leg at the start of this experiment and had to be removed. One sow was observed in heat 110 days after being bred. According to an examination of the reproductive tract she had been cycling normally.

TABLE 3. RESULTS DURING LACTATION

Items Compared	Experiment 1		Experiment 2				Experiment 3	
	Lot 1 Control	Lot 2 +10% Alfalfa	Lot 1 Control	Lot 2 +10% Alfalfa	Lot 3 +3% Fish Sol.	Lot 4 +"B_{12}" Conc.	Lot 1 Control	Lot 2 +10% Alfalfa
Number of sows	2	4	5	5	4[1]	4	3[2]	6[3]
Av. daily feed per sow, lb.	3.1	7.3	10.5	10.2	10.1	8.9	6.5	7.1
Av. daily creep feed per pig, lb.[4]	—	—	0.35	0.18	0.40	0.41	—	—
Av. no. of pigs weaned per litter	3.5	6.5	6.4	5.6	8.2	7.0	4.0	5.5
Av. wt. per pig at 56 days, lb.[5]	19.0	24.5	25.2	23.4	26.9	26.2	21.9	20.7
Av. wt. per litter at 56 days, lb.[5]	66.5	159.0	161.0	130.8	222.5	183.2	87.7	113.7
Feed required per lb. of gain, lb.[6]	8.02	5.30	4.87	4.88	4.43	4.73	5.02	5.45
Percentage of pigs:								
Dead at birth	0	17	7	14	3	9	5	8
Died 1–3 days	0	6	2	3	0	3	18	11
Died 4–21 days	0	0	2	0	0	3	0	15
Died 22–56 days[5]	0	3	12	8	8	0	5	4
Weaned	100	74	76	76	89	85	71	62

[1] One sow farrowed during a period of very cold weather and 9 of her 10 pigs died from exposure. Her data was included in gestation summary only.

[2] Data from 2 sows omitted since they refused to eat and let down milk after farrowing thus causing their pigs to starve.

[3] Lactation data from one sow omitted since she was not eating well and was constipated during the lactation phase. After completion of lactation phase she was operated upon and an obstruction of hair and feces was removed from her duodenum.

[4] Pigs received creep feed from an average age of 28 days to weaning.

[5] Experiment 3 was terminated at 35 days instead of 56 days.

[6] Calculated on the basis of feed consumption and gains from time dams were started on experiments until they weaned their litters. Includes gains of sows and their pigs but not the gains of pigs which died.

In Experiment 3 after the sows had completed one gestation and lactation phase, the number of services per conception for sows that farrowed was 1.4 in Lot 1 (control) and 1.0 in Lot 2 (control plus 10 percent dehydrated alfalfa meal). For all sows started on Experiment 3, the number of services per conception was 2.8 for Lot 1 and 1.1 for Lot 2. Since the sows had already been on purified diets for 233 days at the time Experiment 3 started the previous nutritional treatment may have influenced the conception rate in this experiment.

Table 4 shows the results of slaughter data from sows at the completion of Experiment 3. The 2 sows that lost their litters by starvation

TABLE 4. OVULATION RATE AND APPEARANCE OF REPRODUCTIVE TRACTS OF SOWS SLAUGHTERED IN EXPERIMENT 3

Items Compared	Lot 1 Control	Lot 2 +10% Alfalfa
No. of reproductive tracts observed	5	6
Av. number of corpora lutea	13.0±2.45	13.0±3.52
No. anemic and infantile appearing tracts	1	2
Cystic ovary (20 mm.)	1	0
Fibrous tumor on ovary	0	1

appeared normal and were included in this phase of the experiment. The ovulatory rate of the 2 groups of sows was the same based on the number of corpora lutea. The number of abnormalities observed in the reproductive tracts was fairly equally divided between the 2 treatments.

After the data were collected, it appeared that there was an association of rate of gain of the sow during gestation with litter size. This could not be subjeced to valid statistical test because this question was not included in planning the experiment. It will be investigated in future tests.

In Experiment 1, dehydrated alfalfa meal in the diet apparently increased the voluntary feed intake of sows during lactation since the sows consumed an average of 7.3 lb. per day, whereas the sows on the control diet consumed 3.1 lb. per day. Differences of this magnitude were not observed in Experiments 2 and 3.

Table 5 summarizes the average gestation and lactation performance of the Lot 1 and the Lot 2 sows for all three experiments.

The Lot 2 sows farrowed 1.4 more pigs per litter than the Lot 1 sows. The birth weight and strength of pigs born alive were approximately the same. Although Lot 2 sows farrowed a higher percentage of stillborn pigs they still had more live pigs at birth than Lot 1 sows.

Eighty-eight percent of the Lot 2 sows raised their litters to weaning as compared to 55 percent for Lot 1 sows. The Lot 2 sows had 1.8 more live pigs per litter at farrowing and only 0.7 more pigs at weaning than Lot 1 sows. The lots consumed similar amounts of feed and lost about the same amount of weight during lactation. Sows and litters on

TABLE 5. GESTATION AND LACTATION RESULTS ON CONTROL VS. CONTROL PLUS 10 PERCENT DEHYDRATED ALFALFA MEAL, ALL THREE EXPERIMENTS

Items Compared	Lot 1 Control	Lot 2 +10% Alfalfa
Gestation		
No. of sows started on test	18	17
No. of litters	12	16
Av. number of pigs per litter	7.2	8.6
Av. birth weight per pig, lb.	2.94	2.88
Percentage of pigs farrowed:		
Strong	77	75
Medium	10	9
Weak	6	4
Farrowed alive but strength not known	1	1
Dead	6	11
Lactation		
No. litters	10	15
Av. daily feed per sow, lb.	7.3	7.4
Av. loss in weight per sow	52	53
Feed per lb. total gain	5.22	5.15
Av. number pigs per litter at farrowing	6.6	8.3
Av. number pigs weaned per litter	5.1	5.8
Av. weaning weight per pig, lb.	23.6	22.7
Av. litter weaning weight, lb.	120.1	131.5
Percent pigs weaned	78	70

the two treatments required approximately the same amounts of feed per unit of gain. The litter weaning weights of the dehydrated alfalfa meal sows averaged 10.4 lb. more than for the control sows but when these data were analyzed by fitting constants no significant difference was found.

The results obtained with dehydrated alfalfa meal in these experiments are somewhat in disagreement with previous reports. Ross *et al.* (1942) obtained twice as many pigs that weighed 25 percent more at weaning from sows fed alfalfa meal than from sows fed the control diet. Our data agree quite well with Cunha *et al.* (1944) whose control pigs weighed more at weaning than pigs from sows fed alfalfa meal but a higher percentage of the alfalfa sows farrowed and they weaned more

pigs than the control sows. Fairbanks *et al.* (1945) reported that sows fed alfalfa meal weaned a higher percentage of pigs than the control sows. Krider *et al.* (1946b) reported a higher percentage survival and heavier pigs weaned from sows supplemented with alfalfa meal than those from control sows. Heidebrecht *et al.* (1948) using alfalfa silage instead of alfalfa meal obtained results similar to Krider *et al.* (1946b). Ensminger *et al.* (1946, 1951) obtained heavier pigs at weaning when 15 percent alfalfa meal was added to a purified diet.

TABLE 6. ANALYSIS OF VARIANCE FOR LITTER WEANING WEIGHTS OF EXPERIMENT 2 OMITTING LOT 2

Source of Variation	d/f	Mean Square
Total	12	—
Treatment	2	4412.1
Error	10	1175.3

$$F=\frac{4412.1}{1175.3}=3.75,\ F_{.05}=4.10$$

In Experiment 2, the creep feed data indicated that the pigs in each lot consumed about the same amount of feed except in Lot 2 (control plus 10 percent dehydrated alfalfa meal) where the feed consumption was approximately one-half that of the other lots. This may have been characteristic of the dehydrated alfalfa meal used. Kodras *et al.* (1951) observed that alfalfa leaves depressed growth to a greater extent than stems. Scott (1954) has shown that the concentration of the depressing factor varies with the stage of maturity. Heywang and Bird (1954) found that 0.20 percent of saponin extract from alfalfa caused a growth inhibition in the chick.

Effect of Menhaden Fish Solubles or "Vitamin B_{13}" Concentrate

Table 6 gives the analysis of variance for litter weaning weights of Experiment 2 with Lot 2 data omitted. The data from Lot 2 were included in an over-all analysis to compare the value of adding 10 percent dehydrated alfalfa meal to a purified diet.

Table 6 indicates that the treatment differences approach significance at the 5 percent level. According to table 3, menhaden fish solubles litters weighed 61.5 lb. more than control litters at weaning and this difference approached the 5 percent level of significance.

The response to fish solubles is similar to that in other reports, but greater differences reported by others may be attributed to vitamin B_{12} furnished by the fish solubles. Krider *et al.* (1946c) obtained heavier

weaning weights when the ration was supplemented with sardine fish solubles but poorer weaning weights with menhaden fish solubles. Krider *et al.* (1946a) obtained a higher percentage survival and heavier pigs weaned from sows supplemented with 3 and 6 percent fish solubles than from control sows. Heidebrecht *et al.* (1949) obtained similar results with gilts but yearling sows weaned heavier but slightly fewer pigs per litter.

From the results obtained in these experiments it appears that a successful purified diet has been developed for gestation-lactation experiments. It is advisable to use a protein source low in sulfite to minimize destruction of vitamins.

Summary

A purified diet was used to compare sources of unidentified factors for swine during gestation and lactation.

Gestation and lactation performances of sows fed the purified control diet or this diet supplemented with 10 percent dehydrated alfalfa meal were compared in three experiments. Three samples of dehydrated alfalfa meal were used. Dehydrated alfalfa meal did not have any consistent effect upon litter weaning weight. However, 88 percent of the alfalfa-fed sows weaned litters as compared to 55 percent of the sows fed the control diet. After the sows had been on the diets 233 days, 29 percent fewer services per conception were required by alfalfa-fed sows, although after 448 days there was no difference in ovulatory rate or number of abnormalities of the reproductive tract.

There was no clear-cut evidence that menhaden fish solubles or a "vitamin B_{13}" concentrate possessed factors beneficial to gestation or lactation performance of the sow. However, litters from sows fed 3 percent menhaden fish solubles weighed 61.5 lb. more than control litters at weaning and this difference approached the 5 percent level of significance.

Dehydrated alfalfa meal depressed creep feed consumption of the suckling pig.

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THE EFFECTS OF VARIOUS SEQUENCES OF FULL AND LIMITED FEEDING ON THE REPRODUCTIVE PHENOMENA IN CHESTER WHITE AND POLAND CHINA GILTS[1, 2, 3]

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THAT certain reproductive phenomena in swine can be altered by varying the quantity of feed has been indicated by Robertson *et al.* (1951b), Christian and Nofziger (1952), and Burger (1952). Burger influenced the age and weight at puberty by a severe restriction of the feed intake. Both Robertson *et al.* and Christian and Nofziger reported higher ovulation rates and lower embryo survival rates with a "high" level of feeding than with a "moderate" one, indicating that a level favorable to ovulation was not favorable to embryo survival.

The present experiment is an attempt to establish a sequence of full and limited feeding that will increase litter size by combining the increased number of ova brought about by full-feeding with the increased embryonic survival brought about by limited feeding. Specifically, the effects of different sequences of full and limited feeding upon ovulation rate, embryo survival, and other related phenomena are determined in two different breeds. Possible physiological bases for the different effects of the two feed levels are sought in the associations the different phenomena may have with rate of gain and fatness of the animal.

Materials and Methods

Gilts were obtained from the University Chester White (CW) and Poland China (PC) herds for two experimental trials and approximately equal numbers of the two breeds were assigned randomly to treatment in dry-lot experiments as, individually, they reached 72 days of age in trial I and 70 days of age in trial II.

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Trial I

The 64 gilts (32 CW and 32 PC) in trial I were farrowed during the period from April 20 to May 22, 1952. The oldest gilts were put on experiment July 1 and when these oldest gilts were 4½ months of age (September 7) daily heat checks were begun with a vasectomized teaser boar and were continued until the last gilt was slaughtered on April 15, 1953. The first observed estrus was October 27—50 days after checking with the boar began. The age at first heat was noted for all gilts. They were observed for first estrual cycle length and then bred during the first 24 hours of the second heat.

Gilt weights and feed weigh-back were taken every 14th day (the 154-day weights used in the analysis were obtained from these weights by interpolation).

One-half of the gilts in each of the two breeds was assigned to be slaughtered 24-48 hours after the end of the second heat ("3-day gilts") to furnish data on ovulation at first and second heat and fertilization at second heat. Corpora lutea from both heats were counted for the ovulation information and fertilization data were obtained by flushing the ova from the oviduct or uterine horn with a physiological saline solution and examining the eggs under a low power binocular microscope. The presence of two or more blastomeres of approximately equal size was used as the criterion of fertilization. The other half of the gilts was assigned to be slaughtered on the 25th day after breeding ("25-day gilts"), unless they returned to heat before that time, for determination of the number and normality of embryos and number of second-heat corpora lutea. An embryo was considered normal if it had no gross abnormalities nor apparent degeneration of its tissues. Gilts that returned to heat before the 25th day of gestation were bred back to the boar to which they were originally assigned and then slaughtered 3 days later for ova recovery and examination of the reproductive tract.

The 64 gilts were divided into 8 lots, with two lots comprising a treatment group. Each lot contained two 3-day gilts and two 25-day gilts of each breed.

Four CW and four PC boars from the University herd were used in these studies and the eight gilts in each lot were assigned for mating so that each boar would breed one gilt in each pen. One 3-day gilt and one 25-day gilt selected at random of each breed was bred to a boar at random of the opposite breed. Beyond this the remaining gilts in each breed in each pen were mated at random to boars of their breed. Repeated fertility checks were made on these boars during the course

of the experiment; they were being bred concurrently to gilts in both the 3-day and 25-day category.

Each experiment may be considered as divided into 3 phases: prepuberal period (phase I); first estrual cycle (phase II); and first 25 days of gestation (phase III). Four lots received feed *ad libitum* during phase I; the remaining 4 lots were hand-fed a level approximately ⅔ that concurrently received by the full-fed gilts. Two of the lots that were full-fed during phase I continued to receive feed *ad libitum* throughout the experiment and two of the lots that were limited-fed during phase I were continued on the limited level throughout the trial. Two other lots of gilts full-fed during phase I were changed to limited-feeding during phases II and III, and two other lots of gilts limited-fed during phase I were changed to full feeding during phases II and III. The 4 groups thus established (two lots comprising a group) will be referred to as FFF (full-fed throughout the experiment); FLL (full-fed during phase I and limited-fed during phases II and III); LFF (limited-fed during phase I and full-fed during phases II and III); and LLL (limited-fed throughout the experiment).

A high protein ration (20 percent) was fed *ad libitum* in self-feeders to the full-fed lots and by hand twice daily to the limited-fed lots. Typical feed consumption during the breeding season was 7.1 lb. daily for the full fed and 4.3 lb. for the limited fed. As each lot approximated 125 lb. average weight the entire lot was shifted to a lower protein level (15 percent) for the duration of the experiment—see table 1 for rations used.

In those lots where gilts were designated to be changed in their level of feed, i.e., from full to limited or vice versa, each gilt was changed individually to a pen of the scheduled feeding level as she reached puberty. Extra pens were provided for this purpose.

Back fat measurements were made on the split chilled carcass at the level of the 1st, 7th, and last rib and at the last lumbar vertebra. The values expressed in this report are arithmetic means of these 4 values and are expressed in millimeters.

The data for each phenomenon were examined statistically by the analysis of variance and the analysis of covariance techniques (Snedecor, 1950). Regression and correlation coefficients within breed-feeding groups were calculated and where possible pooled for the two years. In some cases where the nature of the data prevented pooling of the two years, the probabilities of the two trials were combined to test the significance of the findings (Fisher, 1950).

Trial II

In general the procedures were the same as outlined under trial I, with the following exceptions.

The 74 gilts (37 CW and 37 PC) used in this trial were farrowed during the period of April 14 to May 4, 1953.

TABLE 1. RATIONS USED IN THESE STUDIES

Component	Trial I		Trial II	
	20% Protein	15% Protein	18% Protein	13% Protein
Ground corn	480	565	535	615
Ground oats	240	285	250	290
Soybean oil meal (43%)	130	70	...	...
Soybean oil meal (41%)	...	...	100	40
Tankage (50%)	140	70	...	...
Tankage (60%)	...	...	100	40
Ground limestone	6.5	6.5	6.5	6.5
Salt	5.0	5.0	5.0	5.0
Aurofac	2.5	2.5	2.5	2.5

Each gilt was treated for round worms with a 0.75 percent sodium fluoride feed mixture and sprayed for external parasites with benzene-hexachloride immediately preceding assignment to treatment.

Trial II contained only "25-day" gilts. The gilts were divided for the studies on age and weight at puberty, into 8 lots (two lots constituting a treatment group); 6 lots containing 9 gilts each and two lots containing 10 gilts each.

Heat checks were begun September 1 and continued until the experiment was terminated March 2, 1954. The first observed estrus was October 3—33 days after checking was begun.

Semen collections were made and fertility was established on all boars used in trial II by mating them to "tester" non-experimental gilts before they were bred to experimental gilts. Subsequent semen checks were made during the course of the experiment. Additional fertility checks were made throughout the experiment by rebreeding those gilts that had returned to third heat to the same boar.

Each gilt was weighed as she reached 154 days of age.

Early in the course of trial II it was decided that the first 8 gilts with normal reproductive organs to yield information on all the characters being studied in each of the 8 breed-feeding groups would be used in the embryo survival studies.

In trial II 4 feeding-level groups were established: FFF—full-fed throughout the experiment; FFL—full-fed during the prepuberal phase (phase I), full-fed during the first estrual cycle (phase II), and limited-fed during the first 25 days of gestation (phase III); LFL—limited-fed during phase I, full-fed during phase II, and limited-fed during phase III; and the LLL—limited-fed throughout the experiment.

At the time the experiment was terminated, one CW and two PC gilts had not demonstrated estrus. Autopsy revealed an infantile tract in one PC gilt while the other PC gilt had corpora lutea, luteinized follicles, and corpora albicantia. The CW gilt had corpora lutea and corpora albicantia. These gilts were all over 300 days of age when the experiment was terminated and for the purpose of analysis their age and weight at slaughter were taken as the age and weight at puberty.

Results and Discussion

Age at Puberty

Age at puberty for the 64 gilts in trial I ranged from 174 to 297 days, with a mean of 215.7 and a gross standard deviation of 29.2. The 74 gilts in trial II had a mean of 213.2 days with individual gilts ranging from 170 to over 323 days, and a gross standard deviation of 27.8. Neither the means nor the variances differed significantly between the two years.

The trial I CW mean of 225 days differed from the PC mean of 206 days ($P<0.01$), but the CW mean of 211 days did not differ from the PC mean of 216 days in trial II (table 2). The breed variances did not differ significantly in either year. Greater variability of puberal age in some breeds and in some inbred lines than in others was observed by Robertson *et al.* (1951a and 1951b) and by Warnick *et al.* (1951), respectively. Hawk *et al.* (1954) reported that dairy heifers afflicted with scours were significantly older at puberty. The CW-FFF gilts in trial I had numerous cases of scours during the prepuberal phase; the incidence of scouring seemed to be lowest in the PC gilts and in the limited-fed lots. Thus the data of the present study and the work of Robertson *et al.* indicate that unless some disturbing factor, i.e., scouring or some other unknown, exerts an influence, the age at which these two breeds reach puberty should not differ greatly.

Full-fed gilts were significantly older at puberty in both trials than the limited-fed gilts ($P<0.01$). The means and standard deviations in trial I were 223 and 30.6 days for the full-fed and 208 and 21.1 for the limited-fed group; in trial II they were 222 and 33.5 days for the

full-fed group and 205 and 16.3 days for the limited-fed group (table 2). The difference between the variances of the two levels of feeding approached significance in trial I and was significant in trial II ($P<0.02$).

The interaction of breed and level of feeding was not significant in either trial.

The difference between full- and limited-fed groups suggests that "fatness" of the animal, as conditioned by the level of feeding, may

TABLE 2. AVERAGE AGES AND WEIGHTS AT PUBERTY OF GILTS, BY BREED-TREATMENT GROUPS [1]

Breed	Comparison	Trial I			Trial II		
		Full-Fed	Limited-Fed	Total or Average Trial I	Full-Fed	Limited-Fed	Total or Average Trial II
Chester White	Number of gilts	16	16	32	19	18	37
	Age at puberty, da.	237	212	225	218	204	211
	Wt. at puberty, lb.	223	161	192	213	143	179
Poland China	Number of gilts	16	16	32	18	19	37
	Age at puberty, da.	209	204	206	226	206	216
	Wt. at puberty, lb.	198	170	184	220	161	190
Both Breeds	Age at puberty, da.	223	208	216	222	205	213
	Wt. at puberty, lb.	211	166	188	216	152	184

[1] Standard deviations within breed-feeding groups: Trial I—27.9 days, Trial II—26.9 days.

affect the attainment of puberty. This trend is in the same direction as that observed by Christian and Nofziger (1952) but is not in agreement with the findings of Robertson *et al.* (1951b). It possibly agrees with the observations by Phillips and Zeller (1943) so far as fatness is concerned in that the fatter small type Poland China was several days older than the larger type Poland China at puberty. Burger (1952) on the other hand retarded the age at puberty in gilts significantly by limiting the feed intake, but his restrictions were quite severe as compared to the present study. Engle *et al.* (1937) and Asdell and Crowell (1935) delayed the age at puberty of rats by restricting feed intake, but their restriction also was probably quite severe and this appears to be one explanation for the difference in the findings of these groups of workers and the data of the present study.

The association of 154-day weight and age at puberty was highly significant and negative in trial I and negative although not significant in trial II. These correlation coefficients did not differ, so a pooled correlation was calculated and found to be highly significant ($r=-0.27$; $P<0.01$). Negative correlations between 154-day weight and age at puberty were reported within various breeds and breeding groups by both Robertson *et al.* (1951a) and Warnick *et al.* (1951).

However, the latter workers observed a significant positive relationship between inbred lines; those lines which were heavier at 154 days reached puberty later than the lines that were lighter at 154 days of age. This observation is similar so far as effect of group rate-of-gain is concerned to the observations in the present study where the full-fed group in trial I averaged 6 lb. more at 154 days than the limited-fed (120 vs. 114) and were 15 days older at puberty (223 vs. 208). In trial II the full-fed group was 35 lb. heavier at 154 days of age (136 vs. 101) and were 17 days older at puberty 222 vs. 205).

No significant relationship existed in either year between the day of birth in the farrowing season and age at puberty. This does not confirm the reports by Robertson *et al.* (1951a and 1951b), Wiggins *et al.* (1950), and Warnick *et al.* (1951), but most of their studies were conducted on gilts farrowed over a longer period of time than in the present studies. A 32-day period occurred in trial I and a 20-day period in trial II, neither of which allowed adequate appraisal of the effects of time of farrow on age at puberty.

Weight at Puberty

The gilts in trial I ranged from 123 to 293 lb. and had an average weight at puberty of 188 lb. Those in trial II ranged from 105 to over 365 lb. with an average of 184 lb. (table 2). The variation within the two trials did not differ significantly (SD 40 and 52 lb. respectively).

A significant interaction ($P<0.05$) between breed and feeding level occurred in trial I but not in trial II (table 2).

There was a difference between feeding levels within breeds in trial I but a difference between breeds in only the full-fed group. In trial II the means of the two breeds did not differ, but the 216-lb. mean of the full-fed gilts was significantly greater ($P<0.01$) than the 152-lb. mean of the limited-fed gilts and the variation within the full-fed gilts was greater ($P<0.02$) than in the limited-fed gilts; the standard deviations were 51 and 27 lb. respectively.

Length of First Estrual Cycle

The average length of estrual cycle over both trials was 21.1 days. The means for the two breeds did not differ in either of the two trials, but the CW gilts had significantly more variation in each trial than the PC gilts (SD for the CW gilts were 1.6 and 1.6 days and for the PC gilts were 0.9 and 1.0 for the two years respectively). This agrees with earlier work at this station (Robertson *et al.*, 1951a and 1951b).

Comparison of Ovulation Rate at First and Second Heat

Information on the ovulation rate at both first and second heat was provided by the 32 3-day gilts in trial I. The data give an over-all impression of a higher rate on the CW than the PC gilts and also of an increase from the first to the second heat, both of which are in accord with earlier published observations. The experimental treatment in the present study, however, brought out some notable exceptions.

TABLE 3. CHANGE IN OVULATION RATE FROM FIRST TO SECOND HEAT [a]

Initial [b] Level (Phase I)	Final [c] Level (Phase II)	Heat Period	Poland China (PC) Average Ovulation Rate	Poland China (PC) Average Increase (2nd–1st)	Chester White (CW) Average Ovulation Rate	Chester White (CW) Average Increase (2nd–1st)
Full feed	Full Feed (FFF)	1st	11.50[d]	0.5	12.25	3.25
		2nd	12.00		15.50	
	Limited Feed (FLL)	1st	10.25	0.5	13.50	−2.00
		2nd	10.75		11.50	
Limited feed	Full Feed (LFF)	1st	10.25	1.5	9.75	5.50
		2nd	11.75		15.25	
	Limited Feed (LLL)	1st	8.75	0.75	12.25	0.75
		2nd	9.50		13.00	

[a] Data for 1st and 2nd heat obtained from the same 32 "3-day gilts."
[b] Level of feeding from 70 days of age to puberty.
[c] Level of feeding during first estrual cycle.
[d] Each value represents an average of 4 gilts.

There were significantly different increases of 3.25 ova in the CW (table 3) and of 0.5 in the PC when gilts were maintained on full feed from 70 days of age through the second heat. When gilts were kept on limited feed through the second heat, however, the increases were 0.75 ovum in both breeds.

Changes in feed level at the time of puberty brought out marked breed differences. The PC-FLL gilts showed the same increase (0.5 ovum) as the PC-FFF gilts. On the other hand the CW-FLL gilts decreased 2.0 ova in contrast to the 3.25 ova increase of the CW-FFF group—a net difference of 5.25 ova (3.25 minus −2.0). The PC-LFF gilts gave an increase of 1.5 ova as contrasted to a 0.75 ovum increase of the PC-LLL group—a net difference of 0.75 ovum. CW-LFF gilts increased 5.50 ova as compared to a 0.75 ovum increase of the CW-LLL gilts—a net differ-

ence of 4.75 ova. Thus it appears that the PC gilts are relatively stable in the changes in their ovulation rate due to sexual age; the CW gilts, however, show material fluctuations with changes in level of feed intake.

Second Heat Ovulation Rate

Information on second heat ovulation rates was provided by 32 3-day gilts and 25 25-day gilts in trial I and by 53 of the 64 25-day gilts in trial II (table 4). Seven of the original 32 25-day gilts in trial I either

TABLE 4. SECOND HEAT OVULATION RATES BY TRIAL AND BY BREED-FEEDING GROUPS

Trial	Feed Level	Chester White (CW)	Poland China (PC)	Both Breeds
I	F[a]F[b]F[c]	15.1 (7)[d]	12.3 (8)	13.9 (15)
	FLL	10.8 (6)	11.3 (7)	11.1 (13)
	LFF	15.0 (8)	12.0 (7)	13.6 (15)
	LLL	12.6 (7)	9.6 (7)	11.1 (14)
II	FFX[e]	15.4 (12)	11.8 (12)	13.6 (24)
	LFL	15.6 (8)	11.1 (7)	13.5 (15)
	LLL	12.3 (6)	10.2 (8)	11.1 (14)

[a] Denotes level of feeding from beginning of experiment to puberty.
[b] Denotes level of feeding during first estrual cycle.
[c] Denotes level of feeding during first 25 days of gestation.
[d] Values in parenthesis indicate number of animals contributing to the mean of that group.
[e] The FFX group represents the FFF and FFL groups.

returned to their third heat before the 25th day of gestation or their corpora lutea had regressed. In trial II 11 gilts returned to heat before the 25th day of gestation.

Ovulation rates for individual gilts in trial I varied from 7 to 20 ova with a mean of 12.4 and a standard deviation of 2.6; in trial II they varied from 8 to 20 ova with a mean of 12.9 and a standard deviation of 3.0.

The mean ovulation rate of 13.5 ova in the trial I CW gilts was significantly greater ($P<0.01$) than the 11.3 mean of the PC gilts (table 4) as was the 14.8 mean of the trial II CW gilts over the 11.2 mean of the PC gilts. There was no indication of feeding level and breed interaction effects in either trial.

Considering feeding-level over both breeds, the trial I FFF gilts (13.9 ova) had a significant advantage ($P<0.01$) over the LLL group (11.1 ova) and the FLL group (11.1 ova) but did not differ from the LFF group (13.6 ova). In trial II the FF (L and F) (13.6) was significantly ($P<0.01$) greater than the LLL mean (11.1) but did not

differ from the LFL mean (13.5). Thus the short period of full feeding prior to second heat (approximately 3 weeks) of the trial I LFF and the trial II LFL group raised the ovulation rate at second heat to approximately the same level as that produced by full feeding throughout phases I and II. This suggests that large amounts of nutrients are not needed during prepuberty (phase I) to nourish developing follicles. It therefore seems desirable to explore the possibility of shortening the period of full feeding still further to establish the minimum; perhaps only the latter half of the estrual cycle would still produce a maximum ovulation rate.

The association within groups of second heat ovulation rate and rate of gains during phase II was significant when considered over the two years ($r=0.24$; $P<0.05$). Weight at second heat however was insignificantly associated with second-heat ovulation rate in both trials. Thus the relation between current rate of gain and ovulation rate due to gilt individuality seems to be similar to that set up by feeding between groups.

Fat content of the blood as determined by the method of Allen (1934 and 1938) was studied on a preliminary basis on part of the trial I gilts and on all the trial II gilts to test the effects of different sequences of feeding levels and to determine its association with ovulation rate in these two breeds of gilts. Analysis of the data indicates a negative relationship of fat level in the blood and ovulation rate within groups of gilts. The association between groups was inconclusive and present indications are that there is no difference in the blood fat levels of continuously full-fed and continuously limited-fed gilts, but a change from limited to full may increase the fat level of the blood significantly.

Fertilization Rates

Thirty of the 32 3-day gilts of trial I provided information on fertilization rates. Two gilts, one with an abnormal cervix and one improperly served, were not included in the fertilization analysis.

The mean fertilization rate for all gilts was 96 percent. This mean was obtained by summing the percentages of fertilization for the individual gilts and dividing by the total number of gilts involved.

There was no significant difference between the 95 percent fertilization rate for gilts bred to boars of their own breed and the 97 percent rate for gilts bred to boars of a different breed. The individual CW boars ranged from 91 percent to 100 percent and the PC boars ranged from 89 to 98.5 percent without significant difference between boars

or between breed of boar. When the gilts were classified by treatment groups over both breeds, i.e., FFF, FLL, LFF, and LLL, there was no significant difference in the fertilization rate between groups.

The fertilization rates in this study agree quite well with previous studies of fertilization rates in swine at this station (Robertson *et al.*, 1951a, 96 percent; 1951b, 93 percent; Warnick *et al.*, 1951, with inbred gilts and boars—82 percent; Baker *et al.*, 1953, 92.5 percent) and elsewhere (Christian and Nofziger, 1952, 84.5 percent; Wise and Robertson, 1953, 95 percent; Hauser *et al.*, 1952, 94 percent).

That breeding during the first day of heat will give high fertilization rates has been reported by Robertson *et al.* (1951a) and Burger (1952). However, Weaver and Bogart (1943) suggested that in instances where only one service was performed "better results may be expected if it occurs near the end rather than at the beginning of estrus." Gilts in the present study were checked for heat once daily and were bred as soon as heat was detected (mating occurred during the first 24 hours of the second heat period), and as reported earlier, Self *et al.* (1953), the observed fertilization rate was 96 percent.

The improper service of a gilt mentioned above and not included in the fertilization data was one of the 5 incidences of "bladder-breeding" observed in this study. Four other 3-day gilts showed evidence of urethral and/or bladder penetration by the penis of the boar, and one of these also had gelatinous material in the urinary bladder at autopsy, however, known satisfactory services were performed subsequently at the same heat in all four cases. These aberrant matings constituted approximately 16 percent of the 32 3-day gilts; indicating that this may be a source of lowered conception rate when one-time "hand-coupling" matings are practiced. Squiers *et al.* (1952) increased conceptions by 23 percent by mating gilts twice in the same heat period. Weaver and Bogart (1943) increased the conception rate by 18 percent when they mated gilts twice during a heat period. The value of 16 percent for the incidence of bladder breeding in the present study is not far different from the values of 18 percent and 23 percent for increase in conception rate reported by these two groups. Where two services per heat period is practiced the probability of at least one of the services being normal should be increased.

Embryo Survival

Embryo survival was studied in one-half the trial I gilts and in all the trial II gilts. It is expressed as the percentage of corpora lutea in

an individual gilt which were represented by normal embryos at the 25th day of gestation. Embryo survival in these studies ranged from zero to 100 percent with an average of 44.3 percent for trial I and 59.4 percent for trial II.

In trial I 30 of the 32 25-day gilts actually provided embryo survival data. Two gilts were not included in the analysis because of genital abnormalities (a CW gilt had bilateral pyosalpinx and a PC gilt had severe bilateral infundibular-ovarian adhesions). Twenty-one of these gilts had normal embryos at the time of autopsy; 6 gilts had no embryos and had not returned to heat and 3 gilts returned to heat before the 25th day of gestation. These latter 3 gilts were each rebred to the same boar to which they were first mated and then slaughtered 3 days later for examination of the genital tract. Fertilized ova were recovered from 2 of the 3 tracts. The third tract had only unfertilized eggs and no apparent barrier to fertilization was noted. The ovarian structures varied in the 6 gilts which did not have embryos and had not returned to heat. Some of the ovaries contained fully functional corpora lutea and others had corpora lutea in various stages of regression. The corpora lutea in one gilt had regressed completely and the ovaries contained a full compliment of follicles of apparent preovulatory size.

As noted under materials and methods, the first 8 gilts with complete embryo survival data in each group of trial II were to be used in the embryonic death studies. The CW-LLL group, with complete embryo survival data on only 7 gilts, was the only one in which there were insufficient data to obtain the minimum number of gilts. One gilt in this group did not return to second heat and another had a segment of one uterine horn missing. Of the 63 gilts studied, 26 CW and 26 PC gilts had normal embryos. The 5 CW gilts without normal embryos all returned to heat, were rebred and slaughtered 3 days later. Fertilized ova were recovered from all 5 and no abnormalities of the tracts were noted. Five of the 6 PC gilts returned to heat and were rebred. The PC gilt that did not return to heat had 7 degenerating embryos at the time of autopsy. Three of the 5 gilts rebred at the third heat had fertilized ova at autopsy; 1 of the 5 had only unfertilized ova with no apparent tract abnormalities, and no ova could be recovered from the fifth tract.

There was no significant difference between the percent survival (40.8 vs. 47.9) or in embryo numbers (5.33 vs. 5.07) of the trial I CW and PC gilts respectively (table 5), nor between the survival rates of the two breeds in trial II but the number of embryos surviving to

TABLE 5. NUMBER OF CORPORA LUTEA, EMBRYOS, AND PERCENT SURVIVAL BY BREED-FEEDING GROUPS

Breed	Comparison	Trial I				Trial II			
		F[a]F[b]F[c]	FLL	LFF	LLL	FFF	FFL	LFL	LLL
Chester White	Number CL	14.67[2]	9.5[3]	14.75	12.0	15.71[6]	15.0[4]	15.62	12.33[5]
	No. embryos	3.50	3.67[3]	5.75	7.0	9.88	5.75	10.25	9.25[6]
	% survival[1]	25	40[3]	39	58	62	38	68	75[6]
Poland China	Number CL	12.5	12.0	12.3[3]	9.22	12.29[6]	11.0[4]	11.14[6]	10.25
	No. embryos	4.25	6.75	4.33[3]	5.75	5.25	3.50	8.38	8.38
	% survival	39	56	38[3]	56	43	32	74	85
Both Breeds	Number CL	13.6	10.7	13.5	10.8	14.0	13.0	13.53	11.14
	No. embryos	3.89	5.70	5.14	6.37	7.56	4.62	9.32	8.81
	% survival	32	49	38	57	53	35	71	80

[a] Denotes level of feeding from beginning of trial to puberty (phase I).
[b] Denotes level of feeding during first estrual cycle (phase II).
[c] Denotes level of feeding during first 25 days of gestation (phase III).
[1] % survival is the percent of the 2nd heat CL that are represented by normal embryos at the 25th day of gestation.
[2] Each value represents 4 gilts in trial I and 8 gilts in trial II unless otherwise noted.
[3] Average of 3 gilts.
[4] Average of 5 gilts.
[5] Average of 6 gilts.
[6] Average of 7 gilts.

the 25th day was greater ($P<0.05$) in the trial II CW gilts than in the PC gilts (8.8 vs. 6.4)—a reflection of the higher ovulation rate in the CW gilts.

The effects of continued full or continued limited feeding can be observed for trial I by comparing the FFF gilts in table 5 (32 percent embryo survival and 3.89 embryos) with the LLL group (57 percent survival and 6.37 embryos). The 2.48 embryos advantage of the LLL group was not significant; neither was their advantage of 25 percentage points in embryo survival. In trial II the comparison of the FFF and LLL groups over both breeds (table 5) also failed to show a significant difference between the averages of 7.56 embryos in the FFF gilts and the 8.81 embryos in the LLL group; the difference in percent survival between these two groups, however, was highly significant ($P<0.01$).

Pooling the probabilities of the differences in litter size between full and limited feeding for the two years yielded a significant value ($P<0.02$); indicating that despite the lower ovulation rate ordinarily associated with limited feeding a constant regimen of restricted feeding maintained a sufficiently greater percent of the embryos to result in larger litters.

Gilts in trial I that were changed from full to limited feeding at the time of puberty (FLL in table 5) had an advantage of 17 percentage points in embryo survival as compared to the FFF group (49 percent vs. 32 percent) while those changed from limited to full (LFF) had a disadvantage of 19 percentage points (38 vs. 57) as compared to their LLL controls ($P\cong0.33$).

The effects on embryo survival of reducing the feeding level at the time of breeding can be observed for trial II by comparing the FFL with the FFF group in table 5. The difference between the means (4.6 vs. 7.6) approached significance ($P\cong0.05$), and the difference in percent survival was significant ($P<0.05$). That the increased embryonic death rate in the FFL group was not the direct result of the change from full to limited feeding *per se* but was brought about by some other action can be seen by comparing the FFL group with the LFL group. A change from full to limited feed at second heat was made in both groups with the only difference in the feed sequence being the prepuberal level of feeding. The ovulation rates of the two groups did not differ but the difference in percent survival (35 vs. 71) and in number of embryos (4.6 vs. 9.3) at the 25th day were both significant ($P<0.05$).

Full-feeding for only a short period of time (approximately 3 weeks) tended to lower the percentage embryo survival in the LFL as compared

with the LLL but it was more than overcome by the increased ovulation rate which it induced. This particular succession of feeding levels resulted in the greatest number of normal embryos at the 25th day of gestation (table 5).

Pooling the correlation coefficients between rate-of-gain (immediately before and immediately after breeding) and embryo survival for the two trials gave highly significant values (phase II r of 0.45; phase III r of 0.35). Individual gilts treated alike that gained fast immediately prior to breeding or immediately after breeding maintained a higher embryo survival rate than their poorer doing mates. This is partly in agreement with the observation of McKenzie (1928) that gilts which gained faster for the first month after breeding produced larger litters than gilts which gained very little or lost weight during the immediate post-breeding period.

The association of second heat ovulation rate and embryo survival was negative in both years and when the two correlations were pooled showed an association (r of -0.25) which was very near to significance ($P \cong 0.05$). If this phenomenon is real then its adverse effects on litter size seem not to be in proportion to the beneficial effects of increased ovulation rate.

General Discussion

The present studies indicate no clear-cut differences between the means of the PC and CW breeds in age and weight at puberty, fertilization rate, first estrual cycle length, or embryo survival rate; however the CW gilts are more variable in the length of the first estrual cycle and the sexual-age changes in their ovulation rate are affected more by differences in feed level. In general they appear to be superior in ovulation rate and number of normal embryos.

Continuous full feeding was superior to continuous limited feeding for ovulation rate but was inferior for percent embryo survival and number of embryos. Short periods of either full or limited feeding just prior to breeding exerted an effect on the 25-day litter size in the direction of continuous feeding on the same level; ovulation rate was more susceptible to alteration than embryo survival. The effects on final litter size of continuing a level of feeding beyond the 25th day have not been determined.

The evidence of a low positive correlation within the breed-feeding groups of both rate of gain and back fat thickness with ovulation rate as well as the existence of a strong association of these two factors

with ovulation rate between the feed-level groups, and a significant negative association in both cases with embryo survival, raises the question of whether it is the fatness of the animal or the level of available nutrients that is important in determining ovulation rate and survival rate in these gilts.

First with regard to ovulation rate a comparison of the different groups at second heat makes it relatively clear, nutrition-wise, that it is the level of available nutrients. Either full feeding or limited feeding for only 3 weeks was approximately as effective in controlling ovulation rate as similar feeding for a long period of time; i.e., the LF group approximated the FF gilts and the FL gilts approximated the LL gilts, yet these groups were markedly different in fatness.

The correlation of ovulation rate with back fat thickness within groups was 0.06 and with weight was 0.16 and of back fat thickness with weight 0.75. The actual fatness of the animal is probably best indicated by the thickness of back fat with body weight held constant. Using ovulation rate as the dependent factor and slaughter weight and back fat thickness as the independent factors, standard partial regression coefficients were derived. The standard partial regression from back fat thickness to ovulation rate was negative ($p_{FO}=-0.15$; $P\cong 0.33$) while the path from slaughter weight to ovulation rate was positive ($p_{WO}=0.26$; $P\cong 0.09$). Thus, what relationship there is suggests that for a given thickness of back fat the heavier and the growthier the animal the greater the ovulation rate. If we assume that the faster gaining animals within a feeding group (approximately equal feed intake) are the ones that are making gains requiring the least nutrients per unit of gain (gains made up primarily of bone, muscle and connective tissue), and thus have the greatest nutrient surplus for stimulating ovulation then the positive correlation of ovulation rate with rate of gain, the positive standard partial regression on weight and the negative partial regression on back fat thickness all become consistent. Further the association of these factors between individuals within groups becomes consistent with the associations between feeding groups, all on the basis of the level of available nutrients being of major importance.

A similar type of analysis was made for the relationships with embryo survival. The correlation with weight was 0.59 and with back fat thickness was 0.35. It will be recalled that between the groups it was the fatter ones that had the lowest survival. The path from back fat thickness to embryo survival was negative ($p_{FS}=-0.18$; $P\cong 0.20$) and the path from weight at slaughter to embryo survival was positive

(p_{ws}=0.68; $P<0.01$). The negative standard partial regression on back fat thickness within the groups is also consistent. Furthermore the positive standard partial regression on weight suggests that it is the growthier, less fat animals that have the best survival. These data appear to be consistent with the observation of Hetzer and Brier (1940) who reported a significant reduction in the litter size of large, medium, and small type Poland China gilts, respectively. Phillips and Zeller (1943) stated that the small type Poland China presented greater difficulty in "maintaining normal reproduction" than the large type Poland China. These latter workers described the large type as having "longer bodies and legs, and not so thick-set or fat" as the "short, thick-bodied, low-set" small type. More recently Runner and Gates (1954) have reported that progeny were obtained from genetically obese mice by restricting the feed intake and maintaining the mice in a "leaner" condition.

Summary

Gilts of the Chester White (CW) and Poland China (PC) breeds were subjected to different sequences of feed levels; either self-fed (F) or hand-fed at ⅔ the full-fed rate (L) in each of two years to study the effects on ovulation rates, embryo survival and related reproductive phenomena.

In each of the two years the experimental period began when the gilts were approximately 70 days of age and continued until the 25th day after the second heat period. The total period was divided into three phases: Phase I—prepuberty; phase II—the first estrual cycle; and phase III—from second heat (at which time all gilts were bred) until the 25th day of gestation. The feeding levels for the three successive phases are designated by a letter sequence, e.g., LFF.

The 64 gilts in trial I averaged 215.7 days at puberty (SD of 29.2) and the 74 gilts in trial II averaged 213.2 days (SD of 27.8). Full-fed gilts in trial I and trial II respectively were 223 and 222 days of age at puberty as compared to 208 and 205 days for the limited-fed groups ($P<0.01$). Breed did not have a consistent effect over the two trials.

Weight at 154 days and age at puberty were significantly associated (r of -0.27; $P<0.01$) within groups for the two years. Puberal age and time of birth (within spring season) were not associated.

The first estrual cycle length averaged 21.1 days over both trials. Breed means did not differ but the CW gilts had more variation in each trial than the PC gilts ($P<0.02$).

Fifty-seven gilts in trial I averaged 12.4 (SD of 2.6) at second heat and 53 gilts in trial II averaged 12.9 ova (SD of 3.0). The 13.5 and 14.8 ova means of the CW gilts in the two trials respectively were both greater ($P<0.01$) than the 11.3 and 11.2 ova means of the PC gilts. Trial I gilts kept on full feed throughout (FFF) had significantly more ova (13.9; $P<0.01$) than LLL (11.1) or FLL (11.1) gilts. The FFF group did not differ from LFF gilts (13.6 ova). In trial II the FF (L and F) group ovulation rate of 13.6 ova at second heat was greater ($P<0.01$) than the LLL mean of 11.1 ova but did not differ from the LFL mean of 13.5 ova—indicating the necessity of only a short period of full feeding to stimulate maximum ovulation.

Second-heat ovulation rate and rate of gain during the first estrual cycle (within groups) were significantly associated over the two years ($r=0.24$; $P<0.05$). Weight of gilt at second heat and ovulation rate at that heat were not associated.

The over-all fertilization rate of 96 percent was not affected by the individuality of the boar, breed of boar, mating system, feed level of the gilt, or breed of gilt.

Embryo survival averaged 44.3 percent in trial I and 59.4 percent in trial II. The trial I CW and PC gilts had 40.8 percent and 47.9 percent embryo survival and 5.07 and 5.33 embryos respectively ($P\cong0.05$). Trial II CW gilts had more embryos (8.8; $P<0.05$) than the PC gilts (6.4) but they did not differ in percentage of embryos surviving. Trial I FFF gilts had 32 percent embryo survival and 3.89 embryos as compared to 57 percent survival and 6.4 embryos for the LLL gilts. The trial II FFF gilts did not differ from the LLL gilts in number of surviving embryos (7.6 vs. 8.8) but the difference in percent survival was significant ($P<0.01$). Pooled probabilities of litter size differences for the two years indicated larger ($P<0.02$) litter for the LLL gilts. Trial I FLL gilts had 49 percent survival as compared to 32 percent for FFF gilts and the LFF gilts had 38 percent survival as compared to 57 percent for the LLL group. In trial II, FFF gilts had more embryos (7.6 vs. 4.6; $P<0.05$) and greater survival (53 percent vs. 35 percent; $P<0.05$) than the FFL gilts. The LFL gilts had 71 percent survival and 9.3 embryos and did not differ from the FFF gilts.

Rate of gain both immediately before and immediately after breeding, was associated ($P<0.01$) in a positive direction in both instances with embryo survival but the second heat ovulation rate was negatively associated with survival rate ($r=-0.25$; $P\cong0.05$).

Standard partial regressions indicate a possible negative effect of back fat thickness on ovulation rate and on embryo survival but a positive effect ($P<0.05$) of slaughter weight on those two phenomena.

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RIBOFLAVIN STUDIES WITH PIGS

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SEVERAL investigators have published results of experiments concerning the response of young pigs to riboflavin in the diet. However, there has not been good agreement in the interpretation of the results nor in the riboflavin allowances recommended for weanling pigs. Variations in results may have been due to the different diets fed and to the different experimental conditions encountered. Mitchell *et al.* (1950) showed that environmental temperature affects the riboflavin requirement of the pig. Some of the earlier work reported on the riboflavin requirement of the pig was done before vitamin B_{12} was recognized as an essential nutrient and before antibiotics were used in swine rations.

The objectives of this study were (1) further characterization of riboflavin deficiency symptoms in swine, (2) determination of the riboflavin requirement using purified diets believed to be complete in all known nutrients and containing an antibiotic, and (3) study of the interaction, if any, of riboflavin, vitamin B_{12} and the antibiotic chlortetracycline.

Three experiments were conducted in which synthetic diets of a similar nature were individually fed *ad libitum* in self-feeders in wire screen-bottomed cages. A riboflavin depletion period preceded the experimental treatments in each case.

Experiment 1

Procedure

Eighteen Hampshire pigs from three litters sired by the same boar were used in Experiment 1. The pigs averaged 60 days of age initially. Distribution to the three treatments was made at random from six outcome groups, each containing three pigs of the same sex. There were

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three outcome groups each of barrows and gilts. Three of the outcome groups consisted of littermate pigs; the remaining three contained pigs from two litters.

All pigs were fed the control diet (See table 1) during a two-week depletion period. For seven weeks following this, 0.8 and 1.4 mg. of

TABLE 1. COMPOSITION OF CONTROL DIETS

Ingredients	Experiment	
	1 and 2	3
	Pounds	
Corn starch	63.0	60.9
Vitamin-free casein [1]	17.0	—
Drackett 220 protein [2]	—	18.0
DL-methionine	—	0.6
Dextrose	10.0	10.0
Woodflock	3.0	3.0
Corn oil	3.0	3.0
Vitamin A and D oil (3000A–600D)	0.5	0.5
Mineral mixture #2 [3]	4.0	4.0
Vitamin mixture [4]	+	+
Antibiotic [5]	+	—

[1] By assay contained 0.28 mg. riboflavin per pound. Control diet in Experiments 1 and 2 was calculated to contain 0.05 mg. riboflavin per pound.

[2] By assay contained 0.68 mg. riboflavin per pound. Control diet in Experiment 3 was calculated to contain 0.12 mg. riboflavin per pound.

[3] The percentage composition was as follows: 65.0 $CaHPO_4$, 16.0 iodized NaCl, 14.0 K_2CO_3, 3.43 $MgCO_3$, 1.00 $FeSO_4 \cdot xH_2O$, 0.30 $MnSO_4 \cdot H_2O$, 0.10 $CoCl_2 \cdot 6H_2O$, 0.10 $CuSO_4$, 0.02 NaF, 0.04 $ZnCO_3$ and 0.01 KI.

[4] Vitamins added in milligrams per pound of ration: 1.5 thiamin HCl, 15.0 calcium pantothenate, 20.0 niacin, 0.5 folic acid, 1.5 pyridoxine HCl, 0.015 vitamin B_{12}, 0.03 biotin, 10.0 p-aminobenzoic acid, 90.0 inositol, 5.0 a-tocopherol acetate, 0.9 2-methyl-1, 4-naphthoquinone and 600 choline chloride. Riboflavin and vitamin B_{12} were omitted from the control diet in Experiment 3.

[5] In Experiments 1 and 2, chlortetracycline HCl was added at the rate of 5 mg. per pound of diet.

crystalline riboflavin were added per pound of diet for treatments 2 and 3 respectively, and the pigs on treatment 1 were continued on the control diet.

The mean daily temperature in the experimental area during the test was 71° F. (80° F. mean daily maximum and 62° F. mean daily minimum) and the mean relative humidity was 81 percent.

One pig each on treatments 2 and 3 died during the 7-week treatment period. Autopsy showed bilateral pyelonephritis in one pig and necrosis of the liver in the other. One pig on treatment 2 was quite sick and gained slowly during the last three weeks of the test period and it was thought proper to omit this pig in the analysis of the data.

Results

The pigs averaged 39 pounds at the start of the riboflavin depletion period of two weeks. During this period they averaged 1.54 lb. of diet consumed per head daily, 0.33 lb. daily gain and 4.62 lb. of diet per lb. gain.

Final weights at the end of the 7 week treatment period were analyzed by covariance to adjust for differences in initial weight, in weight at the end of the depletion period, and in feed consumption, but only feed consumption proved valuable as an independent variate. The analysis of final weight (W) and feed consumed (F) is given in table 2.

TABLE 2. ANALYSIS OF FINAL WEIGHT (W) AND FEED CONSUMPTION (F)

Source of Variation	d/f	W^2	WF	F^2	d/f	Adjusted W^2
Groups	5	147*	243	528	5	60
Treatment 2 vs. treatment 3	1	42	49	57	1	28
Treatment 1 vs. others	1	9851**	13390	18210**	1	665**
Error	7	27.7	34.7	280	6	27.3

* Significant at 5% level.
** Significant at 1% level.

Final weights and feed consumption of pigs on treatments 2 and 3 did not differ significantly but were highly significantly greater than for the control pigs. When adjusted for feed consumption, final weights of pigs on treatments 2 and 3 were still significantly (P less than 0.01) above those on treatment 1. Adjusted daily gains for treatments 1, 2 and 3 were 0.12, 1.58 and 1.39 lb. respectively.

Blood samples were collected two days before the end of the test. A summary of the blood analyses is given in table 3. There was obviously no significant difference in the items measured.

During the test, symptoms of riboflavin deficiency soon became evident among the control pigs. Lowered feed consumption by the control pigs was noticed throughout the test. Adjusted consumption per pig daily during the 7-week test period was 1.71, 3.42 and 3.50 lb. for treatments 1, 2 and 3, respectively.

The control pigs appeared gaunt with dry, rough skins and coarse hair coats (See figure 1). Considerable scouring was noticed among the control pigs during the last 5 weeks on test but not among pigs on the other two treatments. The control pigs showed increased irritability and hyperactivity.

The six control pigs and several pigs from treatments 2 and 3 were killed at the end of the test to obtain histological data. The ovaries

from the three control gilts showed collapsed follicles and degenerating ova. The liver was quite fatty in four of the six control pigs. Other observations on the control pigs included debris in the Bowman's capsules and tubules of the kidneys, and erosion of the villi of the intestines with a heavy infiltration of lymphocytes into the epithelium.

TABLE 3. BLOOD DATA FROM PIGS IN EXPERIMENT 1

	Treatment		
	1	2	3
Items Compared	Basal Diet	Basal+0.8 mg. Riboflavin/lb.	Basal+1.4 mg. Riboflavin/lb.
Number of pigs	6	4	5
Hemoglobin, gm. per 100 cc.	12.7	12.8	13.2
Erythrocytes, millions per cmm.	7.77	7.91	8.44
Leucocytes			
Thousands per cmm.	36.2	24.6	27.6
Percentage distribution			
Neutrophiles	59.8	60.2[1]	59.0
Eosinophiles	3.2	4.2	3.5
Basophiles	1.2	0.2	0.3
Lymphocytes	32.2	31.8	34.2
Monocytes	3.6	4.2	3.0

[1] The percentage distribution reported for one pig in this treatment was 60, 3, 0, 35, 5, totaling 103. Thus presumably one of these five average percentages is too high by 0.75, and the total exceeds 100 by a similar amount.

Experiment 2

Procedure

The objective of this experiment was to compare additions of 0.6, 0.8 and 1.0 mg. of riboflavin per pound of control diet for growing pigs. The same equipment and control diet were used as in Experiment 1.

Fifteen Hampshire pigs from two litters and three Duroc littermate pigs were distributed to the three treatments at random from outcome trios of the same sex. The pigs averaged 57 days of age initially.

All pigs were fed the control diet during a 2-week depletion period. For 32 days following, the pigs on treatment 1, 2 and 3 received additions of 0.6, 0.8 and 1.0 mg. of riboflavin per pound of control diet, respectively.

The mean daily temperature in the experimental area during the test was 53° F. (62° F. mean daily maximum and 45° F. mean daily minimum) and the mean relative humidity was 81 percent.

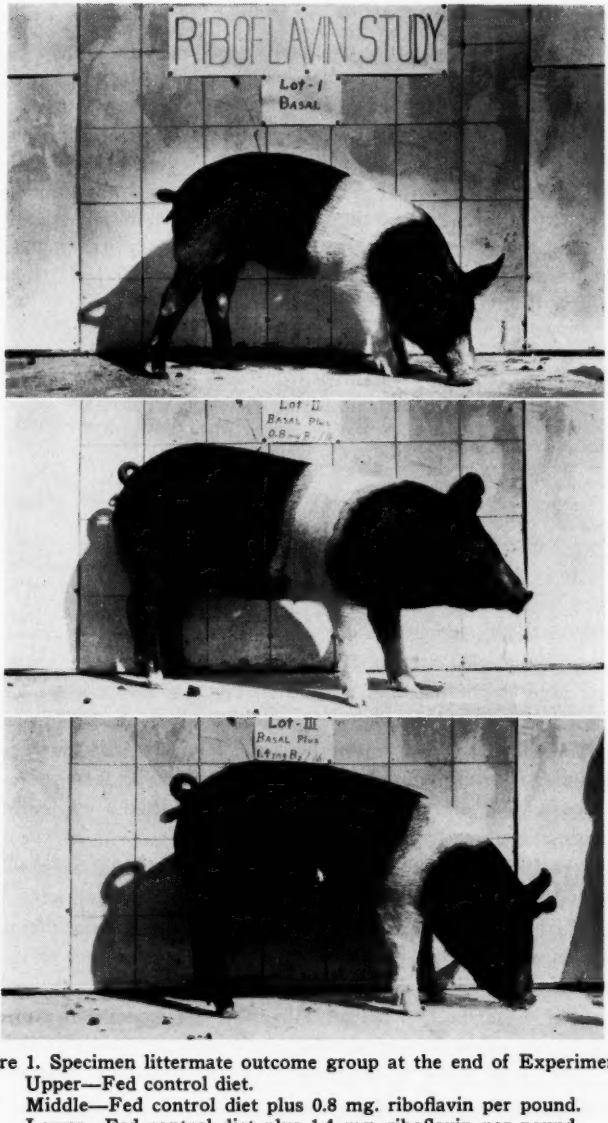

Figure 1. Specimen littermate outcome group at the end of Experiment 1.
Upper—Fed control diet.
Middle—Fed control diet plus 0.8 mg. riboflavin per pound.
Lower—Fed control diet plus 1.4 mg. riboflavin per pound.

Results

The pigs averaged 36 lb. at the start of the depletion period. During the depletion period they averaged 2.08 lb. of diet consumed per head daily, 0.51 lb. daily gain and 4.11 lb. of diet per lb. gain.

Pigs consuming the purified diet containing 0.65, 0.85 and 1.05 mg. of riboflavin per lb. of diet showed similar daily gains and consumed like amounts of feed. Final weight and feed consumption were analyzed by covariance to adjust for differences in initial weight. The analysis is given in table 4. No significant difference was found. Adjusted average daily gains for treatments 1, 2 and 3 were 1.47, 1.45 and 1.47 lb., respectively.

TABLE 4. MEAN SQUARES OF FINAL WEIGHTS (W) AND FEED CONSUMPTION (F), AND THEIR CROSS PRODUCTS, ADJUSTED FOR INITIAL WEIGHT, EXPERIMENT 2

Source of Variation	d/f	W^2	WF	F^2
Outcome groups	5	42	55	91
Treatments	2	10	0	10
Error	9	30	54	127

Experiment 3

Procedure

A factorial design was used in which 32 weanling Hampshire pigs were allotted at random to 8 treatments. The pigs averaged 45 days of age initially. In general, they were available in groups of 4 from the same litter so that 2 litters were needed for one replication and some confounding was necessary. Each of the 3 main effects was confounded in one replication, and 8 pigs from one litter permitted a fourth replication without confounding.

The purified diet shown in table 1 was fed. A suboptimum riboflavin level of 0.4 mg. (control+0.3 mg.) and an optimum level of 1.2 mg. (control+1.1 mg.) of riboflavin was fed with and without 10 mg. of chlortetracycline per pound and with and without 15 mcg. of vitamin B_{12} per pound.

The pigs were fed and managed in the same manner as previously except a one-week depletion period and a 6-week treatment period were used. The mean daily temperature was 54° F. (59° F. mean daily maximum and 48° F. mean daily minimum).

Results

The pigs averaged 29 lb. at the start of the riboflavin and vitamin B_{12} depletion period of one week. During this period they averaged 1.20 lb. of diet consumed per head daily, 0.12 lb. daily gain and 10.3 lb. of diet per lb. gain.

A summary of the statistical analysis of this experiment is given in table 5. Two pigs died during the latter half of the experiment, and

TABLE 5. MEAN SQUARES OF WEIGHTS AFTER THREE AND SIX WEEKS ON TREATMENT, EXPERIMENT 3

	Unadjusted for Feed Consumption				Adjusted for Feed Consumption			
Source of Variation	df	1st 3 wks.	df	6 wks.	df	1st 3 wks.	df	6 wks.
Litters	6	37.28*	6	460.57***	6	12.94	6	8.14
Riboflavin (R)	1	350.21***	1	2231.51***	1	39.34*	1	14.79
Vitamin B_{12} (B)	1	0.07	1	5.06	1	0.30	1	1.29
Chlortetracycline (C)	1	74.73**	1	215.59	1	15.91	1	16.12
RB	1	0.87	1	119.11	1	5.51	1	31.08
RC	1	5.45	1	39.66	1	19.65	1	3.18
BC	1	17.59	1	13.86	1	9.76	1	26.70
RBC	1	17.94	1	230.43	1	12.10	1	3.44
Error	17	10.92	15	75.76	16	8.59	14	22.00

* Significant at 5% level.
** Significant at 2% level.
*** Significant at 1% level.

adjustment was made by means of dummy variates. Also, both final weight and feed consumption were adjusted for differences in initial weight.

The lower level of riboflavin was clearly suboptimal. The difference in feed consumption and in final weight was highly significant after 3 weeks and after 6 weeks on treatment. However, when adjusted also for differences in feed consumption, the difference in final weight was significant at only the 5 percent level after 3 weeks and not significant after 6 weeks. This indicates that a major portion of the pig's response to riboflavin is related to voluntary feed intake. Pigs on the low level of riboflavin were unthrifty but severe deficiency symptoms were not evident.

Chlortetracycline caused an increase in feed consumption and in final weight (each adjusted for differences in initial weight) each significant at the 5 percent level after 3 weeks on treatment, but neither significant after 6 weeks. The difference in final weight after 3 weeks became non-significant when also adjusted for differences in feed consumption.

There was no significant interaction except that for feed consumption, the interaction of riboflavin, vitamin B_{12} and chlortetracycline barely reached the 5 percent significance level after 6 weeks on treatment, but this is considered to be probably merely a result of the vagaries of sampling.

Discussion

The necessity of riboflavin in the nutrition of the pig was demonstrated by Hughes (1939). Later Hughes (1940) concluded that the minimum requirement of riboflavin for the growing pig lies between 1 and 3 mg. per 100 lb. of pig daily. Assuming that the 50 lb. pig consumes 6.4 lb. of feed per 100 lb. of body weight daily (Beeson *et al.* 1953), this would be equivalent to 0.2 to 0.5 mg. per pound of diet.

Krider *et al.* (1949) reported the results of adding 0.5, 0.75 and 1.5 mg. of riboflavin per lb. of a purified ration fed to 30-lb. pigs, and of adding 0.25, 0.5 and 1.0 mg. of riboflavin per lb. of a practical ration fed to 38-lb. pigs. They concluded that 1.4 mg. of riboflavin per lb. of ration appeared to be the practical minimum level for weanling pigs fed in drylot.

Mitchell *et al.* (1950) studied the effect of temperature on the riboflavin requirement of the growing pig. They reported that at 54° F. the requirement was between 0.32 and 0.54 mg. per lb. of diet based on body weight gains and the percentage of neutrophiles in the total leucocytes. They found that at 42° F. the requirement was 1.04 to 1.27 mg. per lb. when based on percentage of neutrophiles but 0.59 to 0.82 mg. per lb. when based on body weight gains.

Miller and Ellis (1951) reported that 0.55 mg. of riboflavin per lb. of diet was inadequate for growing swine, whereas 0.83 mg. was adequate.

Working with baby pigs, Forbes and Haines (1952) concluded that the riboflavin requirement was between 0.68 and 0.91 mg. per lb. of diet at an environmental temperature of 85° F. and 70 percent relative humidity. They reported that the percentage of neutrophiles in the blood was not correlated with riboflavin level in the diet, in contrast to the findings of Mitchell *et al.* (1950) that the percentage of neutrophiles was a sensitive index of the riboflavin adequacy of the diet.

Miller *et al.* (1954) concluded that the riboflavin requirement of the baby pig approximates 1.36 mg. per lb. of diet.

The results of Experiment 1 show that levels of 0.85 and 1.45 mg. of riboflavin per lb. of diet were clearly adequate for normal growth

and feed consumption, but the control diet containing 0.05 mg. per lb. significantly reduced gains and feed consumption. Blood data failed to confirm the findings of Mitchell *et al.* (1950) that the percentage of neutrophiles increases in riboflavin-deficient pigs, agreeing with the findings of Forbes and Haines (1952). Furthermore, the percentage of neutrophiles (59 to 60 percent) for pigs on Experiment 1 agrees well with those reported by Forbes and Haines (53 to 56 percent); whereas Mitchell *et al.* (1950) reported values of 11 to 20 percent for pigs fed adequate riboflavin.

The results of Experiment 2 indicate clearly that the weanling pig's requirement for riboflavin is no higher than 0.65 mg. per lb. of diet when the mean temperature is 53° F. and the mean relative humidity is 81 percent.

It should be borne in mind that in these experiments the riboflavin was furnished in the diet as crystalline riboflavin. If the riboflavin which occurs in natural feedstuffs is less available to the pig than crystalline riboflavin in a purified diet, the requirement in diets of natural feedstuffs would be larger.

The results of Experiment 3 show that the pig's requirement for riboflavin is higher than 0.4 mg. per lb. of diet. Chlortetracycline significantly affected gains during only the first half of the experiment; whereas vitamin B_{12} did not significantly affect gains during either half of the experiment. Under the conditions of this test vitamin B_{12} and chlortetracycline did not significantly influence the riboflavin requirement of the pig.

On the basis of the results presented here, it appears that the riboflavin requirement of the growing pig lies between 0.4 and 0.65 mg. per lb. of diet when the environmental temperature is about 53° F. There is some overlapping when this range of values is compared with the figures reported by Mitchell *et al.* (1950), Hughes (1940) and Miller and Ellis (1951), being somewhat higher than the former two but lower than the latter. It is lower than the practical minimum value suggested by Krider *et al.* (1949) and the baby pig requirement reported by Forbes and Haines (1952) and Miller *et al.* (1954).

Summary

Three experiments were conducted to gain information concerning the riboflavin requirement of the growing pig. Purified diets of a similar nature were used in all experiments, and all pigs were individually fed *ad libitum* in wire screen-bottomed cages. A riboflavin depletion period

preceded the experimental treatments. In the first experiment, three groups of 6 pigs each received diets containing 0.05, 0.85 and 1.45 mg. of riboflavin per lb. Final weights and feed consumption of pigs on the two higher levels did not differ significantly but were highly significantly greater than for pigs on the low level. When adjusted for feed consumption, final weights of pigs on the higher levels were still significantly (P less than 0.01) above the low level. Adjusted daily gains for the above 3 treatments were 0.12, 1.58 and 1.39 lb., respectively.

In the second experiment, three groups each of 6 pigs received diets containing 0.65, 0.85 and 1.05 mg. of riboflavin per lb. No significant difference was found in final weights or feed consumption. Adjusted average daily gains for the three treatments were 1.47, 1.45 and 1.47 lb., respectively.

The third experiment was conducted to determine the effects of chlortetracycline and vitamin B_{12} supplementation upon the riboflavin requirement of the pig. A suboptimum level of 0.4 mg. and an optimum level of 1.2 mg. of riboflavin were fed with and without 10 mg. of chlortetracycline and with and without 15 mcg. of vitamin B_{12}, each per lb. of diet. The 0.4 mg. level of riboflavin was clearly suboptimal, the difference in final weights due to this factor being highly significant. However, after adjusting for differences in feed consumption the riboflavin effect was not significant, indicating that a major portion of the pig's response to riboflavin is related to voluntary feed intake. Chlortetracycline caused a significant increase in gains during the first half of the experiment, though not significant after adjustment for feed consumption. There was no significant interaction. Thus, vitamin B_{12} and chlortetracycline supplementation did not significantly influence the riboflavin requirement of the pig.

The riboflavin requirement of the growing pig is between 0.4 and 0.65 mg. per pound of diet when the mean environmental temperature is 53° F.

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NEWS AND NOTES

Nominations for the Morrison Award. All nominations in writing are due in the Secretary's office by *June 15*. The rules governing this award are stated in the JOURNAL OF ANIMAL SCIENCE, Vol. 11, pp. 807–809. All nominations must be accompanied by a brief biography of the nominee, a list and reprints of publications concerning the research, and a statement of the importance of this research to the livestock industry and animal science. The cash award for 1955 will be $1,250 and a gold watch.

Nominations for the American Feed Manufacturer's Award. The rules governing this award are as stated in the JOURNAL OF ANIMAL SCIENCE, Vol. 7, pp. 257–258. No formal nominations from the membership are required, although the Award Committee would appreciate receiving nominations. These should also be sent by *June 15* to the Secretary's office. Research published in 1953 and 1954 will be considered for the 1955 award.

1955 Annual Meeting. The annual meeting will be held at Hotel Sherman, Chicago, on November 25 and 26. You will be sent a questionnaire in early June regarding papers and titles which are to be submitted to the Secretary's office by July 20. Abstracts of papers are to be submitted by August 15. Only members of the Society can submit papers, but associate authors can be non-members.

Professor F. B. Morrison of Cornell University has returned from a short visit to Venezuela. During this time Professor Morrison served in an advisory capacity on problems in the field of animal husbandry to Dr. Amando Tamayo, Ministro de Agricultural de Cria, in that country.

Jorge de Alba, head of the Animal Industry Department at the Inter American Institute of Agricultural Sciences in Turrialba, Costa Rica has completed a study for the Government of Colombia on the Native Breeds of Cattle of that Country. A bulletin describing the present status and proposed plans for development of these breeds is available at the Institute.

Zane Palmer has joined the Department of Animal Husbandry and Nutrition at the University of Florida. He will be in charge of teaching and research in Meats.

L. L. Bucy is now in the Department of Animal Husbandry, California Polytechnic Institute, San Luis Obispo, California.

Husdjursraserna. Edited by Ivar Johansson. 1953. LT's Förlag, Stockholm and Grøndahl & Søn, Oslo. 523 pp. 471 figures. (In Swedish and Norse.) The world's races and breeds of livestock and their place in agriculture are described and pic-

tured. The book is divided into eight main sections. The editor wrote the section on species and races and that on swine. Other sections and their authors are: Equines by Johs. Jespersen; cattle by Nils Korkman; sheep and goats by S. Berge; rabbits and fur-bearing animals by Ole Venge; poultry by Johs. Høie; and geographic distribution of animal husbandry by Nils Westermarck. Although intended primarily for use in agricultural schools and by animal producers in the Scandinavian lands, the book is so broadly based and widely inclusive that many in the United States and other countries will find it interesting.—J. L. LUSH.

The American Society of Animal Production will purchase the following back numbers of the JOURNAL OF ANIMAL SCIENCE at $1.00 each:

Volume	Year	Number			
1	1942	1	2	3	4
2	1943	..	2	3	4
3	1944	1	2	3	4
4	1945	1	2	3	4
5	1946	1	2	3	4
6	1947	1	2	3	4
7	1948	1	2	3	4
8	1949	1	2	3	4
9	1950	1	2	..	..
10	1951	1	..	3	..
11	1952	1	2	..	..

Those desiring to sell copies of the JOURNAL should write to Dr. W. M. Beeson, Secretary-Treasurer, Purdue University, Lafayette, Indiana.

The Western Section of the American Society of Animal Production will meet jointly with the Western Division of the American Dairy Science Association for their annual meeting on July 10–13, inclusive at the University of Wyoming, Laramie.

A Poisonous Plant Shortcourse will be offered at the Utah State Agricultural College, Logan, Utah, June 20 to July 1. For further information contact the Department of Range Management.

The Third Inter-American meeting of Livestock Production sponsored by the Food and Agriculture Organization of the United Nations and the Inter-American Institute of Agricultural Sciences was held in Buenos Aires, Argentina, April 18–30, 1955. Among those participating in the technical session were R. W. Phillips and S. H. Work of FAO's Agriculture Division, and H. O. Hetzer of the Animal and Poultry Husbandry Research Branch, ARS, Beltsville, Md.

K. A. Huston has left the Virginia Polytechnic Institute to become Associate Professor of Dairy Husbandry at Kansas State College, succeeding F. E. Eldridge in this position.

W. R. Harvey, who was formerly Associate Professor of Dairy Husbandry at the University of Idaho, has joined the staff of the Dairy Husbandry Research Branch, ARS, Beltsville.

R. E. Ely joined the technical staff of the State Experiment Stations Division, ARS on January 31, and will serve as Agricultural Administrator in the field of animal nutrition. Prior to this assignment he served as Nutritionist in the Dairy Husbandry Research Branch, ARS, Beltsville.

H. A. Stewart former head of the Animal Husbandry section of the Animal Industry Department at North Carolina State College has been made Assistant Director of Research of the North Carolina Agricultural Experiment Station.

Thomas D. Watkins resigned his position as animal fiber technologist in the Animal and Poultry Husbandry Research Branch, ARS, Beltsville, in December 1954, to join the animal husbandry staff of the Texas A & M College, with the rank of professor. His new assignment includes teaching, research and extension activities in sheep and wool.

Louis L. Madsen, Head of the Beef Cattle Research Section, Animal and Poultry Husbandry Research Branch, ARS, Beltsville, Md., and former President of Utah State Agricultural College, has been appointed Director of the Institute of Agricultural Sciences at Washington State College, effective about May 1.

W. N. Smith formerly with the Animal Disease and Parasite Research Branch, USDA, Beltsville, is now Assistant Parasitologist at the University of Georgia.

Robert M. Richard was appointed Assistant Animal Husbandman at the University of Arizona on January 16, 1955. He was formerly on the animal husbandry staff at the North Dakota Agricultural College.

L. R. Arrington has been made Assistant Animal Husbandman at the Florida Agricultural Experiment Station, Gainesville.

T. L. Meade has resigned his position of Assistant Animal Nutritionist at the Everglades Experiment Station, Belle Glade, Florida, to do consulting work for a commercial company.

Ward W. Repp, who received his Doctorate at Iowa State College in 1954, has joined the staff of the Animal Husbandry Department at New Mexico A & M College.

Kenneth G. Jackson has resigned his position of Assistant Animal Husbandman at the University of Tennessee to become Manager of the Howard Johnson Restaurant in Knoxville.

W. E. Neville, Jr., Associate in animal breeding at the Georgia Agricultural Experiment Station, Griffin, is on leave of absence to continue graduate work at the University of Wisconsin.

Wm. York Varney and Richard F. Getz have joined the animal husbandry staff at the University of Kentucky with the rank of Assistant Animal Husbandman.

Barton Farthing has been appointed Research Instructor in the Animal Industry Department of the North Carolina Agricultural Experiment Station.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, doubled-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints ordered at that time.

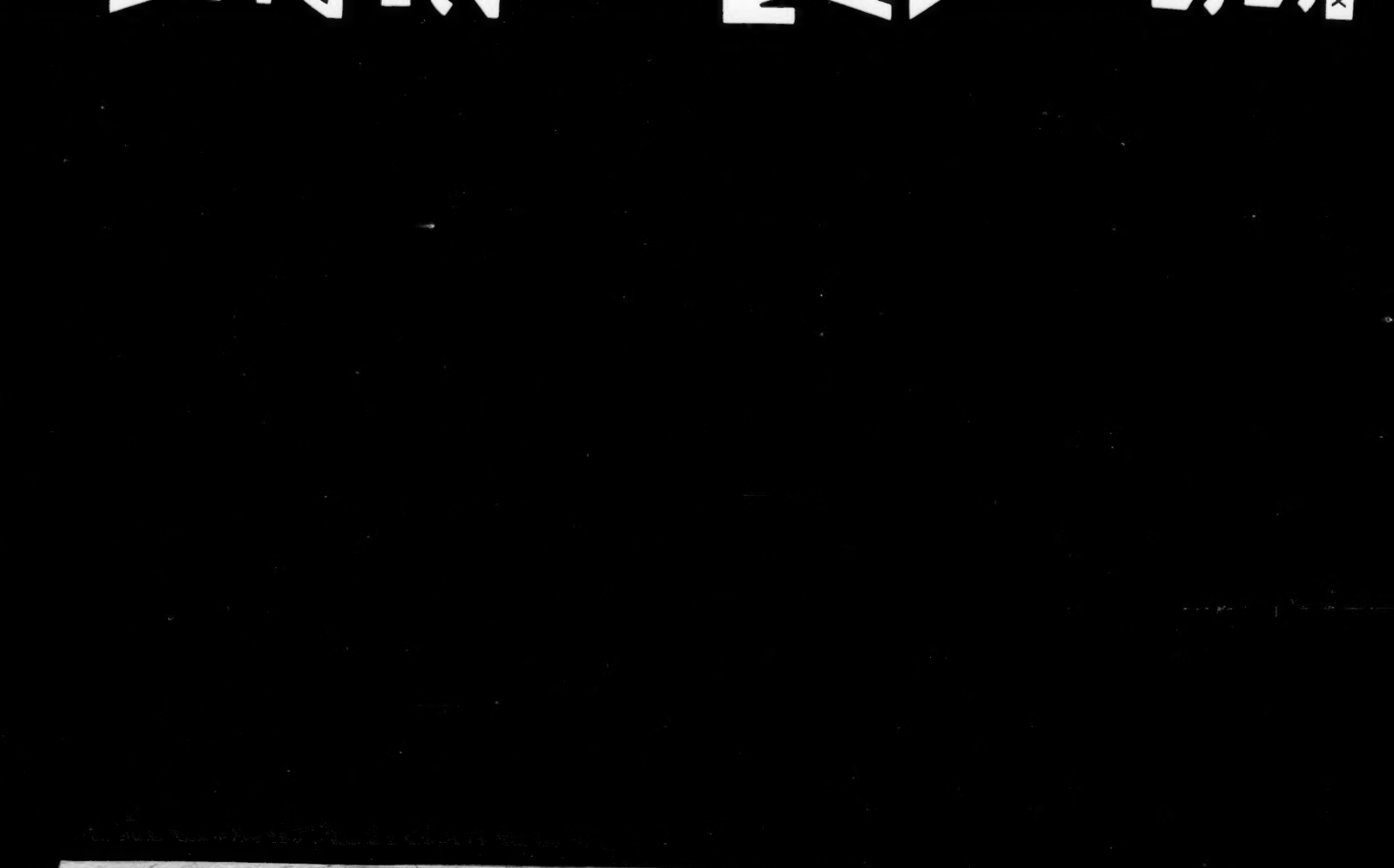
6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts." *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest to members. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. J. K. Loosli, Department of Animal Husbandry, Cornell University, Ithaca, New York, or to the publishers: Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. W. M. Beeson, Department of Animal Husbandry, Purdue University, Lafayette, Ind., or to the publishers, Boyd Printing Company, 372–374 Broadway, Albany 7, New York.